PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

United States Patent and Trademark Office
(Box PCT)
Crystal Plaza 2
Washington, DC 20231
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 29 June 1998 (29.06.98)	
International application No. PCT/AU97/00770	**Applicant's or agent's file reference**
International filing date (day/month/year) 13 November 1997 (13.11.97)	**Priority date** (day/month/year) 13 November 1996 (13.11.96)
Applicant BAXTER, Alan, George	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

12 June 1998 (12.06.98)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO **34, chemin des Colombettes** **1211 Geneva 20, Switzerland**	Authorized officer L. Panakal
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 338.83.38

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF THE RECORDING OF A CHANGE

(PCT Rule 92bis.1 and Administrative Instructions, Section 422)

To:

HUGHES, E., John, L.
Davies Collison Cave
1 Little Collins Street
Melbourne, VIC 3000
AUSTRALIE

Date of mailing (day/month/year)
23 February 1999 (23.02.99)

Applicant's or agent's file reference

IMPORTANT NOTIFICATION

International application No.
PCT/AU97/00770

International filing date (day/month/year)
13 November 1997 (13.11.97)

1. The following indications appeared on record concerning:

[X] the applicant [ ] the inventor [ ] the agent [ ] the common representative

Name and Address
AMRAD OPERATIONS PTY. LTD.
576 Swan Street
Richmond, VIC 3121
Australia

State of Nationality: AU
State of Residence: AU
Telephone No.:
Facsimile No.:
Teleprinter No.:

2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:

[ ] the person [X] the name [X] the address [ ] the nationality [ ] the residence

Name and Address
CENTENARY INSTITUTE OF CANCER
MEDICINE AND CELL BIOLOGY
Missenden Road
Camperdown 2040
New South Wales
Australia

State of Nationality: AU
State of Residence: AU
Telephone No.:
Facsimile No.:
Teleprinter No.:

3. Further observations, if necessary:

4. A copy of this notification has been sent to:

[X] the receiving Office
[ ] the International Searching Authority
[X] the International Preliminary Examining Authority
[ ] the designated Offices concerned
[X] the elected Offices concerned
[ ] other:

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No.: (41-22) 740.14.35

Authorized officer
P. Regis

Telephone No.: (41-22) 338.83.38

P, ENT COOPERATION TREAT

PCT

NOTIFICATION OF THE RECORDING OF A CHANGE

(PCT Rule 92bis.1 and Administrative Instructions, Section 422)

From the INTERNATIONAL BUREAU

To:

HUGHES, E., John, L.
Davies Collison Cave
1 Little Collins Street
Melbourne, VIC 3000
AUSTRALIE

Date of mailing (day/month/year)
17 March 1999 (17.03.99)

Applicant's or agent's file reference

IMPORTANT NOTIFICATION

International application No.
PCT/AU97/00770

International filing date (day/month/year)
13 November 1997 (13.11.97)

1. The following indications appeared on record concerning:

[X] the applicant [ ] the inventor [ ] the agent [ ] the common representative

Name and Address

CENTENARY INSTITUTE OF CANCER
MEDICINE AND CELL BI
LOGY
Missenden Road
Camperdown 2040
New South Wales
Australia

State of Nationality: AU
State of Residence: AU
Telephone No.:
Facsimile No.:
Teleprinter No.:

2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:

[ ] the person [ ] the name [X] the address [ ] the nationality [ ] the residence

Name and Address

CENTENARY INSTITUTE OF CANCER
MEDICINE AND CELL BI
LOGY
Missenden Road
Camperdown 2050
New South Wales
Australia

State of Nationality: AU
State of Residence: AU
Telephone No.:
Facsimile No.:
Teleprinter No.:

3. Further observations, if necessary:

4. A copy of this notification has been sent to:

[X] the receiving Office
[ ] the International Searching Authority
[X] the International Preliminary Examining Authority
[ ] the designated Offices concerned
[X] the elected Offices concerned
[ ] other:

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland
Facsimile No.: (41-22) 740.14.35

Authorized officer
P. Regis
Telephone No.: (41-22) 338.83.38

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF THE RECORDING OF A CHANGE

(PCT Rule 92bis.1 and Administrative Instructions, Section 422)

To:

MONDAY 29 MAR 1999

HUGHES, E., John, L.
Davies Collison Cave
1 Little Collins Street
Melbourne, VIC 3000
AUSTRALIE

Date of mailing (day/month/year)
17 March 1999 (17.03.99)

Applicant's or agent's file reference
195617

IMPORTANT NOTIFICATION

International application No.
PCT/AU97/00770

International filing date (day/month/year)
13 November 1997 (13.11.97)

1. The following indications appeared on record concerning:

[X] the applicant [ ] the inventor [ ] the agent [ ] the common representative

Name and Address
CENTENARY INSTITUTE OF CANCER
MEDICINE AND CELL BIOLOGY
Missenden Road
Camperdown 2040
New South Wales
Australia

State of Nationality: AU
State of Residence: AU
Telephone No.:
Facsimile No.:
Teleprinter No.:

2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:

[ ] the person [ ] the name [X] the address [ ] the nationality [ ] the residence

Name and Address
CENTENARY INSTITUTE OF CANCER
MEDICINE AND CELL BIOLOGY
Missenden Road
Camperdown 2050
New South Wales
Australia

State of Nationality: AU
State of Residence: AU
Telephone No.:
Facsimile No.:
Teleprinter No.:

3. Further observations, if necessary:

4. A copy of this notification has been sent to:

[X] the receiving Office
[ ] the International Searching Authority
[X] the International Preliminary Examining Authority
[ ] the designated Offices concerned
[X] the elected Offices concerned
[ ] other:

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland
Facsimile No.: (41-22) 740.14.35

Authorized officer
P. Regis
Telephone No.: (41-22) 338.83.38

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF THE RECORDING OF A CHANGE

(PCT Rule 92bis.1 and Administrative Instructions, Section 422)

To:

TUESDAY, - 9 MAR 1999

HUGHES, E., John, L.
Davies Collison Cave
1 Little Collins Street
Melbourne, VIC 3000
AUSTRALIE

Date of mailing (day/month/year)
23 February 1999 (23.02.99)

Applicant's or agent's file reference
195861 7

IMPORTANT NOTIFICATION

International application No.
PCT/AU97/00770

International filing date (day/month/year)
13 November 1997 (13.11.97)

1. The following indications appeared on record concerning:

[X] the applicant [ ] the inventor [ ] the agent [ ] the common representative

Name and Address
AMRAD OPERATIONS PTY. LTD.
576 Swan Street
Richmond, VIC 3121
Australia

State of Nationality: AU
State of Residence: AU
Telephone No.:
Facsimile No.:
Teleprinter No.:

2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:

[ ] the person [X] the name [X] the address [ ] the nationality [ ] the residence

Name and Address
CENTENARY INSTITUTE OF CANCER MEDICINE AND CELL BIOLOGY
Missenden Road
Camperdown 2040
New South Wales
Australia

State of Nationality: AU
State of Residence: AU
Telephone No.:
Facsimile No.:
Teleprinter No.:

3. Further observations, if necessary:

4. A copy of this notification has been sent to:

[X] the receiving Office
[ ] the International Searching Authority
[X] the International Preliminary Examining Authority
[ ] the designated Offices concerned
[X] the elected Offices concerned
[ ] other:

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland
Facsimile No.: (41-22) 740.14.35

Authorized officer
P. Regis
Telephone No.: (41-22) 338.83.38

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTICE INFORMING THE APPLICANT OF THE COMMUNICATION OF THE INTERNATIONAL APPLICATION TO THE DESIGNATED OFFICES

(PCT Rule 47.1(c), first sentence)

To:

THURSDAY, - 4 JUN 1998

HUGHES, E., John, L.
Davies Collison Cave
1 Little Collins Street
Melbourne, VIC 3000
AUSTRALIE

Date of mailing (day/month/year)	
22 May 1998 (22.05.98)	
Applicant's or agent's file reference 1958617	**IMPORTANT NOTICE**
International application No. PCT/AU97/00770	**International filing date** (day/month/year) 13 November 1997 (13.11.97)
Priority date (day/month/year) 13 November 1996 (13.11.96)	
Applicant AMRAD OPERATIONS PTY. LTD. et al	

1. Notice is hereby given that the International Bureau has communicated, as provided in Article 20, the international application to the following designated Offices on the date indicated above as the date of mailing of this Notice:
   AU,BR,CA,CN,EP,IL,JP,KP,KR,NO,PL,US

   In accordance with Rule 47.1(c), third sentence, those Offices will accept the present Notice as conclusive evidence that the communication of the international application has duly taken place on the date of mailing indicated above and no copy of the international application is required to be furnished by the applicant to the designated Office(s).

2. The following designated Offices have waived the requirement for such a communication at this time:
   AL,AM,AP,AT,AZ,BA,BB,BG,BY,CH,CU,CZ,DE,DK,EA,EE,ES,FI,GB,GE,GH,HU,ID,IS,KE,KG,KZ, LC,LK,LR,LS,LT,LU,LV,MD,MG,MK,MN,MW,MX,NZ,OA,PT,RO,RU,SD,SE,SG,SI,SK,SL,TJ,TM,TR, TT,UA,UG,UZ,VN,YU,ZW
   The communication will be made to those Offices only upon their request. Furthermore, those Offices do not require the applicant to furnish a copy of the international application (Rule 49.1(a-bis)).

3. Enclosed with this Notice is a copy of the international application as published by the International Bureau on
   22 May 1998 (22.05.98) under No. WO 98/20900

REMINDER REGARDING CHAPTER II (Article 31(2)(a) and Rule 54.2)

If the applicant wishes to postpone entry into the national phase until 30 months (or later in some Offices) from the priority date, **a demand for international preliminary examination** must be filed with the competent International Preliminary Examining Authority before the expiration of 19 months from the priority date.

It is the applicant's sole responsibility to monitor the 19-month time limit.

Note that only an applicant who is a national or resident of a PCT Contracting State which is bound by Chapter II has the right to file a demand for international preliminary examination.

REMINDER REGARDING ENTRY INTO THE NATIONAL PHASE (Article 22 or 39(1))

If the applicant wishes to proceed with the international application in the **national phase**, he must, within 20 months or 30 months, or later in some Offices, perform the acts referred to therein before each designated or elected Office.

For further important information on the time limits and acts to be performed for entering the national phase, see the Annex to Form PCT/IB/301 (Notification of Receipt of Record Copy) and Volume II of the PCT Applicant's Guide.

The International Bureau of WIPO **34, chemin des Colombettes** **1211 Geneva 20, Switzerland**	Authorized officer J. Zahra
Facsimile No. (41-22) 740.14.35	Telephone No. (41-22) 338.83.38

PATENT COOPERATION TREATY

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

PCT

To: Agent :

DAVIES COLLISON CAVE
1 Little Collins Street
MELBOURNE VIC 3000

19
THURSDAY, 18 JUN 1998

NOTIFICATION OF RECEIPT
OF DEMAND

(PCT Rule 61.1(b), first sentence
and Administrative Instructions, Section 601)

Pin up.

Date of mailing *(day/month/year)* 17 JUN 1998 (17/6/98)	
Applicant's or agent's file reference PO3593/EJH 1958617	**IMPORTANT NOTIFICATION**

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/AU97/00770	13 NOV 1997 (13/11/97)	13 NOV 1996 (13/11/96)

Applicant

AMRAD Operations Pty Ltd (et al.)

1. The applicant is hereby notified that this International Preliminary Examining Authority considers the following date as the date of receipt of the demand for international preliminary examination of the international application:

12 JUN 1998 (12/6/98)

2. This date of receipt is:

☒ the actual date of receipt of the demand.

☐ the date on which the proper corrections to the demand were timely received.

3. ☐ This date is **AFTER** the expiration of 19 months from the priority date.

Attention: The election(s) made in the demand does (do) not have the effect of postponing the commencement of the national phase until 30 months from the priority date (or later in some Offices) (Article 39(1)). Therefore, the acts for entry into the national phase must be performed within 20 months from the priority date (or later in some Offices) (Article 22).

For details, see Annex B to Form PCT/IB/301 sent by the International Bureau and Volume II of the PCT Applicant's Guide.

☐ This notification confirms the information given in person or by telephone on:

4. Only where paragraph 3 applies, a copy of this notification has been sent to the International Bureau.

Name and mailing address of the IPEA/AU	Authorized officer
AUSTRALIAN PATENT OFFICE **PO BOX 200, WODEN ACT 2606,** **AUSTRALIA**	(Mrs) Cecilia TRACEY
Facsimile No. 02 6285 3929	Telephone No. (02) 6283 2511

Form PCT/IPEA/402 (July 1992)

PCT

REQUEST

The undersigned requests that the present international application be processed according to the Patent Cooperation Treaty.

International Application No.

International Filing Date

Name of receiving Office and "PCT International Application"

Applicant's or agent's file reference *(if desired) (12 characters maximum)*

Box No. I TITLE OF INVENTION

A METHOD OF TREATMENT AND PHARMACEUTICAL COMPOSITIONS USEFUL FOR SAME

Box No. II APPLICANT

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (i.e. country) of residence if no State of residence is indicated below.)*

AMRAD OPERATIONS PTY LTD
576 Swan Street
RICHMOND 3121
VICTORIA
AUSTRALIA

☐ This person is also inventor.

Telephone No.

Facsimile No.

Teleprinter No.

State *(i.e. country)* of nationality: AUSTRALIA

State *(i.e. country)* of residence: AUSTRALIA

This person is applicant for the purposes of: ☐ all designated States ☒ all designated States except the United States of America ☐ the United States of America only ☐ the States indicated in the Supplemental Box

Box No. III FURTHER APPLICANT(S) AND/OR (FURTHER) INVENTOR(S)

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (i.e. country) of residence if no State of residence is indicated below.)*

BAXTER, Alan George
76 Albion Street
ANNANDALE 2038
NEW SOUTH WALES
AUSTRALIA

This person is:

☐ applicant only

☒ applicant and inventor

☐ inventor only *(If this check-box is marked, do not fill in below.)*

State *(i.e. country)* of nationality: AUSTRALIA

State *(i.e. country)* of residence: AUSTRALIA

This person is applicant for the purposes of: ☐ all designated States ☐ all designated States except the United States of America ☒ the United States of America only ☐ the States indicated in the Supplemental Box

☐ Further applicants and/or (further) inventors are indicated on a continuation sheet.

Box No. IV AGENT OR COMMON REPRESENTATIVE; OR ADDRESS FOR CORRESPONDENCE

The person identified below is hereby/has been appointed to act on behalf of the applicant(s) before the competent International Authorities as: ☒ agent ☐ common representative

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

HUGHES, E John L
SLATTERY, John M
CORBETT, Terence G

DAVIES COLLISON CAVE
1 Little Collins Street
MELBOURNE 3000
VICTORIA
AUSTRALIA

Telephone No. +61 3 9254 2777

Facsimile No. +61 3 9254 2770

Teleprinter No.

☐ Mark this check-box where no agent or common representative is/has been appointed and the space above is used instead to indicate a special address to which correspondence should be sent.

Box No. V DESIGNATION OF STATES

The following designations are hereby made under Rule 4.9(a) *(mark the applicable check-boxes; at least one must be marked)*:

Regional Patent

- ☒ **AP** **ARIPO Patent:** **GH** Ghana, **KE** Kenya, **LS** Lesotho, **MW** Malawi, **SD** Sudan, **SZ** Swaziland, **UG** Uganda, **ZW** Zimbabwe, and any other State which is a Contracting State of the Harare Protocol and of the PCT
- ☒ **EA** **Eurasian Patent:** **AM** Armenia, **AZ** Azerbaijan, **BY** Belarus, **KG** Kyrgyzstan, **KZ** Kazakstan, **MD** Republic of Moldova, **RU** Russian Federation, **TJ** Tajikistan, **TM** Turkmenistan, and any other State which is a Contracting State of the Eurasian Patent Convention and of the PCT
- ☒ **EP** **European Patent:** **AT** Austria, **BE** Belgium, **CH and LI** Switzerland and Liechtenstein, **DE** Germany, **DK** Denmark, **ES** Spain, **FI** Finland, **FR** France, **GB** United Kingdom, **GR** Greece, **IE** Ireland, **IT** Italy, **LU** Luxembourg, **MC** Monaco, **NL** Netherlands, **PT** Portugal, **SE** Sweden, and any other State which is a Contracting State of the European Patent Convention and of the PCT
- ☒ **OA** **OAPI Patent:** **BF** Burkina Faso, **BJ** Benin, **CF** Central African Republic, **CG** Congo, **CI** Côte d'Ivoire, **CM** Cameroon, **GA** Gabon, **GN** Guinea, **ML** Mali, **MR** Mauritania, **NE** Niger, **SN** Senegal, **TD** Chad, **TG** Togo, and any other State which is a member State of OAPI and a Contracting State of the PCT *(if other kind of protection or treatment desired, specify on dotted line)* .....

National Patent *(if other kind of protection or treatment desired, specify on dotted line)*:

- ☒ **AL** Albania .....
- ☒ **AM** Armenia .....
- ☒ **AT** Austria .....
- ☒ **AU** Australia .....
- ☒ **AZ** Azerbaijan
- ☒ **BA** Bosnia and Herzegovina .....
- ☒ **BB** Barbados
- ☒ **BG** Bulgaria .....
- ☒ **BR** Brazil .....
- ☒ **BY** Belarus
- ☒ **CA** Canada
- ☒ **CH and LI** Switzerland and Liechtenstein
- ☒ **CN** China .....
- ☒ **CU** Cuba .....
- ☒ **CZ** Czech Republic .....
- ☒ **DE** Germany .....
- ☒ **DK** Denmark .....
- ☒ **EE** Estonia .....
- ☒ **ES** Spain .....
- ☒ **FI** Finland .....
- ☒ **GB** United Kingdom
- ☒ **GE** Georgia .....
- ☒ **GH** Ghana .....
- ☒ **HU** Hungary .....
- ☒ **IL** Israel .....
- ☒ **IS** Iceland
- ☒ **JP** Japan .....
- ☒ **KE** Kenya .....
- ☒ **KG** Kyrgyzstan .....
- ☒ **KP** Democratic People's Republic of Korea .....
- ☒ **KR** Republic of Korea .....
- ☒ **KZ** Kazakstan .....
- ☒ **LC** Saint Lucia
- ☒ **LK** Sri Lanka
- ☒ **LR** Liberia
- ☒ **LS** Lesotho .....
- ☒ **LT** Lithuania
- ☒ **LU** Luxembourg .....
- ☒ **LV** Latvia
- ☒ **MD** Republic of Moldova .....
- ☒ **MG** Madagascar .....
- ☒ **MK** The former Yugoslav Republic of Macedonia .....
- ☒ **MN** Mongolia
- ☒ **MW** Malawi .....
- ☒ **MX** Mexico .....
- ☒ **NO** Norway
- ☒ **NZ** New Zealand .....
- ☒ **PL** Poland .....
- ☒ **PT** Portugal .....
- ☒ **RO** Romania
- ☒ **RU** Russian Federation .....
- ☒ **SD** Sudan
- ☒ **SE** Sweden
- ☒ **SG** Singapore
- ☒ **SI** Slovenia .....
- ☒ **SK** Slovakia .....
- ☒ **SL** Sierra Leone
- ☒ **TJ** Tajikistan .....
- ☒ **TM** Turkmenistan .....
- ☒ **TR** Turkey .....
- ☒ **TT** Trinidad and Tobago .....
- ☒ **UA** Ukraine .....
- ☒ **UG** Uganda .....
- ☒ **US** United States of America .....
- ☒ **UZ** Uzbekistan .....
- ☒ **VN** Viet Nam .....
- ☒ **YU** Yugoslavia .....
- ☒ **ZW** Zimbabwe

Check-boxes reserved for designating States (for the purposes of a national patent) which have become party to the PCT after issuance of this sheet:

- ☒ ID Indonesia .....
- ☐ .....
- ☐ .....
- ☐ .....

In addition to the designations made above, the applicant also makes under Rule 4.9(b) all designations which would be permitted under the PCT except the designation(s) of _____.

The applicant declares that those additional designations are subject to confirmation and that any designation which is not confirmed before the expiration of 15 months from the priority date is to be regarded as withdrawn by the applicant at the expiration of that time limit. ***(Confirmation of a designation consists of the filing of a notice specifying that designation and the payment of the designation and confirmation fees. Confirmation must reach the receiving Office within the 15-month time limit.)***

See Notes to the request form

Box No. VI PRIORITY CLAIM Supplemental Box ☐

The priority of the following earlier application(s) is hereby claimed:

	Country (in which, or for which, the application was filed)	Filing Date (day/month/year)	Application No.	Office of filing (only for regional or international applications)
item (1)	AUSTRALIA	13 November, 1996 (13-11-96)	P03593	
item (2)				
item (3)				

Mark the following check-box if the certified copy of the earlier application is to be issued by the Office which for the purposes of the present international application is the receiving Office (a fee may be required):

☒ The receiving Office is hereby requested to prepare and transmit to the International Bureau a certified copy of the earlier application(s) identified above as item(s): (1)

Box No. VII INTERNATIONAL SEARCHING AUTHORITY

Choice of International Searching Authority (ISA) *(If two or more International Searching Authorities are competent to carry out the international search, indicate the Authority chosen; the two-letter code may be used.)* ISA / _____

Earlier search *Fill in where a search (international, international-type or other) by the International Searching Authority has already been carried out or requested and the Authority is now requested to base the international search, to the extent possible, on the results of that earlier search. Identify such search or request either by reference to the relevant application (or the translation thereof) or by reference to the search request.*

Country (or regional Office): Date *(day/month/year)*: Number:

Box No. VIII CHECK LIST†

This international application contains the †following number of sheets:

1. request	3	sheets
2. description	20	sheets
3. claims	3	sheets
4. abstract	1	sheets
5. drawings	4	sheets
Total	31	sheets

This international application is accompanied by the item(s) marked below:

1. ☐ separate signed power of attorney
2. ☐ copy of general power of attorney
3. ☐ statement explaining lack of signature
4. ☐ priority document(s) *identified in Box No. VI as item(s):*
5. ☐ fee calculation sheet
6. ☐ separate indications concerning deposited microorganisms
7. ☐ nucleotide and/or amino acid sequence listing (diskette)
8. ☐ other *(specify)*:

Figure No. _____ of the drawings (if any) should accompany the abstract when it is published.

Box No. IX SIGNATURE OF APPLICANT OR AGENT

Next to each signature, indicate the name of the person signing and the capacity in which the person signs (if such capacity is not obvious from reading the request).

AMRAD OPERATIONS PTY LTD
By:

Name: STEWART SHUTE
Position: COMPANY SECRETARY.

BAXTER, Alan George

For receiving Office use only

1. Date of actual receipt of the purported international application:	2. Drawings: ☐ received: ☐ not received:
3. Corrected date of actual receipt due to later but timely received papers or drawings completing the purported international application:	
4. Date of timely receipt of the required corrections under PCT Article 11(2):	
5. International Searching Authority specified by the applicant: ISA/	6. ☐ Transmittal of search copy delayed until search fee is paid

For International Bureau use only

Date of receipt of the record copy by the International Bureau:

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference PO3593/EJH	**FOR FURTHER ACTION**	see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.
International application No. **PCT/AU 97/00770**	International filing date *(day/month/year)* 13 November 1997	(Earliest) Priority Date *(day/month/year)* 13 November 1996

Applicant
(1) AMRAD OPERATIONS PTY LTD
(2) BAXTER, Alan George

This international search report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This international search report consists of a total of **5** sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. [ ] **Certain claims were found unsearchable** (See Box I)

2. [ ] **Unity of invention is lacking** (See Box II)

3. [ ] The international application contains disclosure of a **nucleotide and/or amino acid sequence listing** and the international search was carried out on the basis of the sequence listing

   [ ] filed with the international application

   [ ] furnished by the applicant separately from the international application,

      [ ] but not accompanied by a statement to the effect that it did not include matter going beyond the disclosure in the international application as filed

   [ ] transcribed by this Authority

4. With regard to the **title,**

   [ ] the text is approved as submitted by the applicant.

   [X] the text has been established by this Authority to read as follows:

MYCOBACTERIUM CELL WALL COMPOSITIONS

5. With regard to the **abstract,**

   [ ] the text is approved as submitted by the applicant

   [X] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is:

   Figure No.

   [ ] as suggested by the applicant.

   [ ] because the applicant failed to suggest a figure

   [ ] because this figure better characterises the invention

   [X] None of the figures

International Application No.
PCT/AU 97/00770

Box III TEXT OF THE ABSTRACT (Continuation of item 5 of the first sheet)

The present invention relates generally to a method of immunomodulating therapy and pharmaceutical compositions useful for same. More particularly, the present invention provides a method of ameliorating the effects of autoimmune conditions. Even more particularly, the present invention contemplates a method for preventing, delaying onset of or otherwise ameliorating the effects of insulin-dependant diabetes mellitus (IDDM) by administering a cell wall subunit or a chemical or functional equivalent thereof from *Mycobacterium* or a related organism or other suitable biological source. The present invention is further directed to a pharmaceutical composition useful in preventing, delaying onset of, curing, curing in association with islet replacement or otherwise ameliorating the effects of autoimmune conditions such as IDDM or for enhancing an immune response against melanoma or other cancer comprising a cell wall subunit or a chemical or functional equivalent thereof from *Mycobacterium* or a related organism or other suitable biological source. The cell wall subunit is preferably mycolyl-arabinogalactan-peptidoglycan (MAPG) or a component thereof.

INTERNATIONAL SEARCH REPORT

International Application No.
PCT/AU 97/00770

A. CLASSIFICATION OF SUBJECT MATTER

Int Cl[6]: A61K 39/04, 38/02, C07K 14/35

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC: A61K C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
AU; IPC as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
DERWENT: (MYCOBACTER: or BCG) AND (CELL()WALL or IDDM or DIABET: or AUTOIMMUNE or AUTO()IMMUNE or CANCER); MAPG
CHEMICAL ABSTRACTS: ?PEPTIDOGLYCAN? AND (MYCOLYL or MYCOLYLARABINO); MAPG

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 96/00579 A (LAVESARZNIMITTEL GMBH) 11 January 1996 entire document	1-6, 10-12, 20-22
X	WO 96/26288 A (ADCOCK INGRAM LIMITED) 29 August 1996 entire document	1-17, 20-22
X	WO 94/16727 A (VETREPHARM, INC.) 4 August 1994 entire document	1-6, 10-12, 20-22

[X] Further documents are listed in the continuation of Box C

[X] See patent family annex

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search
18 December 1997

Date of mailing of the international search report
13 JAN 1998

Name and mailing address of the ISA/AU
AUSTRALIAN INDUSTRIAL PROPERTY ORGANISATION
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Authorized officer
T. SUMMERS

Telephone No.: (02) 6283

C (Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages *(Remove spaces when completed if the page is too long)*	Relevant to claim No.
X	WO 87/02249 A (RAGLAND William L) 23 April 1987 entire document	1-6, 10-12, 20-22
X	GB 2120548 A (RIBI IMMUNOCHEM RESEARCH INC.) 7 December 1983 entire document	1-17, 20-22
X	US 4152423 A (ADAM et al.) 1 May 1979 entire document	1-6, 10-12, 20-22
X	Hirschfield GR *et al.*, "Peptidoglycan - Associated Polypeptides of *Mycobacterium tuberculosis*" *J. Bacteriol.* (Feb 1990) 172(2),1005-1013 abstract	1-22
X	Azuma I *et al.*, "Fractionation of Mycobacterial Cell Wall" *J. Bacteriol.* (Nov 1968) 96(5), 1885-1887 entire document	15-19
X	Baxter A.G. *et al.*, "Mycobacteria precipitate an SLE-like syndrome in diabetes-prone NOD mice", *Immunology* (1994) 83, 227-231 abstract and discussion	1-16, 20-22
X	Sadelain M.W.J. *et al.*, "Prevention of Type I diabetes in NOD mice by adjuvant immunotherapy", *Diabetes* (May 1990), 39(5), 583-587 abstract	1-16, 20-22
X	Harada M *et al.*, "Prevention of overt diabetes and insolitis in NOD mice by a single BCG vaccination" *Diabetes Res. Clin. Pract.* (1990) 8, 85-89 abstract	1-16, 20-22
X	Mediline Abstract UI: 93171635, Qin MY *et al.*, "Complete Freund's adjuvant-induced Tcells prevent the developmentand adaptive transfer of diabetes in nonbese diabetic mice", *J. Immunol* (1993), 150(5), 2072-2080 abstract	1-16, 20-22
X	Mediline Abstract UI: 93209598, Chugh IB *et al.*, "Protective efficacy of different cell-wall fractions of Mycobacterium tuberculosis", *Folia. Microbiol (Praha)*, (1992), 37(6), 407-412 abstract	1-16, 20-22
X	Mediline Abstract UI: 83254632, Beaudet R *et al.*, "Stimulation of non-specific anti-tumor resistance in the mouse using cell wall preparations from four BCG substrains",. *Ann. Immunol. (Paris)* (1983), 134C(2), 215-226 Abstract	1-16, 20-22
X	Dockrell HM *et al.*, "Inductiion of Th1 Cytokine Responses by Mycobacterial Antigens in Leprosy", *Infect. Immun..*, (Oct 1996), 64(10), 4385-4389 Abstract	1-16, 20-22
X	McNeil M *et al.*, "Evidence for the Nature of the Link between the Arabinogalactan and Peptidoglycan of Mycobacterial Cell Walls" *J. Biiol. Chem.* (25 Oct 1990) 265(30), 18200-18206	15-19
X	Barnes PF *et al.* "Tumour Necrosis Factor Production in Patients with Leprosy" *Infect. Immun.* (Apr 1992), 60(4), 1441-1446 abstract	1-16, 20-22

INTERNATIONAL SEARCH REPORT
Information on patent family members

International Application No.
PCT/

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report		Patent Family Member					
WO	9600579	AU	28884/95	CA	2194138	CZ	9603753
		DE	4422859	EP	804211	NO	965593
		PL	317892				
WO	9626288	AU	47287/96	CA	2187779	EP	811075
		EP	755517	PL	316792	WO	9528642
WO	9416727	AU	59680/94	BR	9406496	CA	2154689
		CN	1118574	EP	681479		
WO	8702249	AU	65258/86	CA	1293190	EP	238657
		US	4744984				
GB	2120548	AU	14015/83	CA	1202903	NZ	204020
		US	4520019				
US	4152423	AR	192504	AU	48697/72	BE	791472
		CA	1013691	CH	564085	DE	2256838
		ES	408766	FR	2160326	JP	48061619
		LU	66491	NL	7215627	US	4036953
		AU	57069/73	CA	1009972	CH	587346
		FR	2189019	GB	1440842	JP	49066817
		ZA	7304076				

END OF ANNEX

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 1958617/EJH/AF	**FOR FURTHER ACTION**	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416).
International application No. **PCT/AU 97/00770**	International filing date *(day/month/year)* 13 November 1997	Priority Date *(day/month/year)* 13 November 1996
International Patent Classification (IPC) or national classification and IPC **Int. Cl.**[6] A61K 39/04, A61K 38/02; C07K 14/35		
Applicant AMRAD OPERATIONS PTY LTD et al		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of **8** sheets, including this cover sheet.

   ☐ This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of sheet(s).

3. This report contains indications relating to the following items:

I	☒	Basis of the report
II	☐	Priority
III	☐	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
IV	☐	Lack of unity of invention
V	☒	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
VI	☐	Certain documents cited
VII	☐	Certain defects in the international application
VIII	☒	Certain observations on the international application

Date of submission of the demand 12 June 1998	Date of completion of the report 8 March 1999
Name and mailing address of the IPEA/AU AUSTRALIAN PATENT OFFICE PO BOX 200 WODEN ACT 2606 AUSTRALIA Facsimile No. (02) 6285 3929	Authorized Officer **ARATI SARDANA** Telephone No. (02) 6283 2627

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/AU 97/00770

I. Basis of the report

1. With regard to the **elements** of the international application:*

[X] the international application as originally filed.

[ ] the description, pages , as originally filed,
pages , filed with the demand,
pages , filed with the letter of .

[ ] the claims, pages , as originally filed,
pages , as amended (together with any statement) under Article 19,
pages , filed with the demand,
pages , filed with the letter of .

[ ] the drawings, pages , as originally filed,
pages , filed with the demand,
pages , filed with the letter of .

[ ] the sequence listing part of the description:
pages , as originally filed
pages , filed with the demand
pages , filed with the letter of

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.
These elements were available or furnished to this Authority in the following language which is:

[ ] the language of a translation furnished for the purposes of international search (under Rule 23.1(b)).

[ ] the language of publication of the international application (under Rule 48.3(b)).

[ ] the language of the translation furnished for the purposes of international preliminary examination (under Rules 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, was on the basis of the sequence listing:

[ ] contained in the international application in written form.

[ ] filed together with the international application in computer readable form.

[ ] furnished subsequently to this Authority in written form.

[ ] furnished subsequently to this Authority in computer readable form.

[ ] The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

[ ] The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

4. [ ] The amendments have resulted in the cancellation of:

[ ] the description, pages

[ ] the claims, Nos.

[ ] the drawings, sheets/fig

5. [ ] This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).**

* *Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17).*

** *Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report*

V. **Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

1. Statement

Novelty (N)	Claims **4 and 11-14**	**YES**
	Claims **1-3, 5-10 and 15-22**	**NO**
Inventive step (IS)	Claims **4 and 11-14**	**YES**
	Claims **1-3, 5-10 and 15-22**	**NO**
Industrial applicability (IA)	Claims **1-22**	**YES**
	Claims	**NO**

2. Citations and explanations (Rule 70.7)

Citations

a) WO 87/02249 A (Ragland, William L) 23 April 1987
entire document

b) GB 2120548 A (Ribi Immunochem Research Inc) 7 December 1983
entire document

c) US 4152423 A (Adam et al) 1 May 1979
entire document

d) Hirschfield, G R *et al* "Peptidoglycan-Associated Polypeptides of *Mycobacterium tuberculosis*" J Bacteriol (February 1990) 172(2), 1005-1013
abstract

e) Azuma, I *et al*, "Fractionation of Mycobacterial Cell Wall" *J Bacteriol* (November 1968) 96(5), 1885-1887
entire document

f) Baxter, A G *et al*, "Mycobacteria precipitate an SLE-like syndrome in diabetes-prone NOD mice", *Immunology*
abstract and discussion

g) Sadelain, M W J *et al*, "Prevention Type I diabetes in NOD mice by adjuvant immunotherapy", *Diabetes* (May 1990), 39(5), 583-587
abstract

h) Harada, M *et al*, "Prevention of overt diabetes and insulitis in NOD mice by a single BCG vaccination" *Diabetes Res Clin Prac* (1990) 8, 85-89
abstract

Supplemental Box

(To be used when the space in any of the preceding boxes is not sufficient)

Continuation of : V

i) Mediline Abstract UI: 93171635, Qin, M y *et al*, "Complete Freund's adjuvant-induced T-cells prevent the development and adaptive transfer of diabetes in non-obese diabetic mice", *J Immunol* (1993), 150(5), 2072-2080
abstract

j) Mediline Abstract UI: 93209598, Chugh, I B *et al*, "Protective efficacy of different cell-wall fractions of Mycobacterium tuberculosis",*Folia, Microbiol (Praha)*, (1992), 37(6), 407-412
abstract

k) Mediline Abstract UI: 83254632, Beaudet, R *et al*, "Stimulation of non-specific anti-tumor resistance in the mouse using cell wall preparations from four BCG substrains", *Ann Immunol (Paris)* (1983), 134C(2), 215-226
abstract

l) Dockrell, H M *et al*, "Induction of Thl Cytokine Responses by Mycobacterial Antigens in Leprosy", *Infect Immun*, (October 1996), 64(10, 4385-4389)
abstract

m) McNeil, M *et al*, "Evidence for the Nature of the Link between the Arabinogalactan and Peptidoglycan of Mycobacterial Cell Walls" *J Biol Chem* (25 October 1990) 265(30), 18200-18206

n) Barnes, PF *et al*, "Tumour Necrosis Factor Production in Patients with Leprosy", *Infect Immun.* (April 1992), 60(4), 1441-1446
abstract

o) WO 96/00579 A (Lavesarznimittel GmbH) 11 January 1996
entire document

p) WO 96/26288 A (Adcock Ingram Limited) 29 August 1996
entire document

q) WO 94/16727 A (Vetrepharm, Inc) 4 August 1994
entire document

Explanations

The claims of PCT/AU97/00770 are directed to the use of components of the cell wall of Mycobacterium in methods of treatment for autoimmune disease including insulin-dependant diabetes mellitis (IDDM), anti-tumour immune response, melanoma and bladder cancer. Claims 15 and 16 are directed to a composition of matter comprising mycolyarabinogalactan-pepidoglycon (MAPG). Claims 17-19 are directed to methods of isolation of MAPG. Claims 1-14 and 20-22 are directed to methods of immunomodulatory therapy of a mammal using one or more of the components of the cell wall of Mycobacterium which includes MAPG.

WO 87/02249 (Document (a)) describes and claims an antiviral immunotherapeutic agent which comprises an effective amount of deproteinized bacterial cell wall suspension. Claim 2 is directed to bacteria selected from the group consisting of Mycobacterium species. Claim 11 describes a method of isolation of cell walls of bacteria and the preparation of an antiviral immunotherapeutic agent. Example 10 discloses the treatment of herpesvirus infection in horses known as acute rhinopneumonitis, using Mycobacterium cell wall extract.

Supplemental Box
(To be used when the space in any of the preceding boxes is not sufficient)

Continuation of: V

Claims 1-3, 5, 6, 10 and 17-20 lack both novelty and inventive step when compared with the disclosure and claims of WO 87/02249 (Document (a)).

GB 2120548 (Document (b)) discloses and claims a method of isolation of cell wall skeleton from mycobacteria including Mycobacterium bovis. The applicant has isolated a composition containing cell wall skeleton (CWS) and purified trehalose dimycolates (TDM). The composition has been shown to be efficient in obtaining suppression and regression of tumour cells.

Claims 1, 2, 5-10 and 17-22 are not novel and lack an inventive step when compared with the disclosure and claims of GB 2120548 (Document (b)).

US 4152423 (Document (c)) discloses the use of cell wall extracts of Mycobacterium to produce vaccine preparations against bacterial, viral and parasitic infections and antigens for tumours (see column 14 lines 60-68). Materials included in the scope of the present invention are well known for use as adjuvants to increase the efficiency of vaccines, especially if they are weak immunogens. The monomer units of the wall include a mucopeptide combined with a glycolipid containing an arabinogalactan.

Claims 1-3, 5, 7, 8, 10 and 15-20 are not novel and lack an inventive step when compared with US 4152423 (Document (c)).

Hirschfield et al, (1990) (Document (d)) discloses cell wall polypeptides isolated from Mycobacterium. Purified cell walls of Mycobacterium tuberculosis were extracted with sodium dodecyl sulphate to produce an insluble residue composed of the mycolylarabinogalactan-peptidoglycan complex. The publication does not teach the possible uses of the complex in a method of immunomodulatory therapy. However, a person skilled in the art would read the disclosure by Hirschfield et al, (1990) in the light of common general knowledge and find that a claim to the method of immunomodulation would lack an inventive step. Claims 1, 2 and 20 lack an inventive step and claims 15-19 lack novelty when compared with the disclosure of Hirschfield et al. (Document(d)).

Azuma et al, (1968) (Document (e)) teaches the isolation of cell wall components of Mycobacterium tuberculosis. At page 1886, column 2, at the last paragraph work by Kanetsuna is cited which suggests that mycolic acid-arabinoglactan-mucopeptide complex may be a common structure of the mycobacterial cell wall. The publication does not teach a method of treatment. Claims 15-19 lack novelty when compared with the disclosure by Azuma et al.

Baxter et al, (1994) (Document (f)) discloses the use of preparations from Mycobacterium bovis to prevent the development of diabetes in non-obese diabetic mice. There is no disclosure that cell wall components are of use as vaccines. The disclosure does not deprive any of the claims of novelty or inventive step. A similar comment applies to the publication by Sadelain et al, (1997), Harada et al, (1990) and Qin et al, (1993). Claims 1-22 are both novel and have an inventive step when compared with these publications.

Sadelain, MWJ, et al (Document (g)) discloses the treatment of diabetes in non-obese diabetic (NOD) mice. NOD mice were given a single injection of complete Freund's adjuvant. Animals receiving Freund's adjuvant showed microscopic evidence of healthy insulin-secreting cells for up to eighteen months. Evaluation of immune system function in the treated and untreated animals indicate that CFA caused an apparently permanent decrease in the level of T-lymphocytes, a type of cell associated with the progression of diabetes. The results suggest possible treatment for diabetes as well as for other autoimmune disorders.

Supplemental Box
(To be used when the space in any of the preceding boxes is not sufficient)

Continuation of:V

A single injection of Freund's adjuvant (CFA) given at early age prevented the appearance of diabetes and greatly increased the life span of NOD mice without additional therapy. The non-obese diabetic (NOD) mice spontaneously develop an insulin-dependent diabetes mellitis that has many immunological and pathological similarities to human insulin-dependent diabetes. Freund's adjuvant comprises killed Mycobacterium.

Claims 1-4, 10-13, 20 and 21 lack both novelty and inventive step when compared with the disclosure in Document (g).

Harada, M et al, (1990) (Document (h)) discloses that a single intravenous injection of live Bacillus Calmette-Guèrin BCG at ten weeks of age produced a potent suppression of insulitis and overt diabetes in non-obese, diabetes-prone (NOD) female mice(see the abstract). Earlier work disclosing the use of live bacillus Calmette- Guèrin (BCG) against cancers and Leishmania infection (page 85, column 2). Bacillus Calmette- Guèrin (BCG) is also known as Mycobacterium Tuberculosis.

Claims 1-6, 11-13 and 20-22 lack both novelty and inventive step when compared with the disclosure in Document (h).

Qin, HY, et al, (1993) (Document (i)) discloses that mice injected with Mycobacterium tuberculosis or Mycobacterium bovis (BCG) vaccine at four weeks of age were found to be protected from diabetes. Claims 1-4, 11-14, 20 and 21 lack both novelty and inventive step when compared with Document (i).

Chugh et al, (1992) (Document (j)) discloses immunisation with cell wall fractions from Mycobacteria of mycolic acids and arabinogalactans (cell-wall-protein-peptidoglycan complex). Mice immunised with cell wall fractions had increased protection when challenged with Mycobacterium tuberculosis. Claims 1, 2 and 20 lack novelty and inventive step when compared with the disclosure by Chugh et al, (1992).

Beaudet et al, (1983) (Document (k)) describes a study of the anti-tumour activity of cell walls, deproteinized cell walls and cell wall skeleton isolated from four BCG (Mycobacterium) substrains. The preparations were found to offer significant protection against Ehrlich carcinoma but were no protection for L1210 leukaemia. There is no disclosure of a method of treatment of insulin-dependent diabetes mellitis (IDDM) or of isolation of mycolyl-arabinogalactan-peptidoglycan (MAPG). Claims 1, 2, 5, 6, 20 and 22 lack both novelty and inventive step when compared with the publication by Beaudet et al, (1983).

Dockrell et al, (1996) (Document (l)) used mycobacterial antigens including mycolylarabinogalactan peptidoglycan to produce an interferon gamma response in tuberculoid leprosy. A method of isolation of mycolylarabino-galactan peptidoglycan is disclosed. Claims 1, 2, 7, 15, 16 and 20 lack novelty and inventive step when compared with the disclosure by Dockerell et al, (1996).

McNeil et al, (1990) (Document (m)) discloses the isolation and analysis of mycolylarabinogalactan and the peptidoglycan of the cell walls of Mycobacterium bovis, Mycobacterium leprae and Mycobacterium tuberculosis. There is no disclosure of the use of cell wall components in method of treatment. Claims 15-19 lack both novelty and inventive step when compared to McNeil et al.

Barnes et al (1992) studied the effects of mycobacterium on the role of tumour necrosis factor (TNF) in leprosy. Claims 1, 2, 7, 8, 10 and 15-20 lack both novelty and inventive step when compared with Barnes et al, (1992).

Supplemental Box
(To be used when the space in any of the preceding boxes is not sufficient)

Continuation of:V

Barnes, PF et al (1992) (Document (n)) discloses the host response to Mycobacterium leprae. Treatment of human patients with Mycobacterium leprae, mycolylarabinogalactan peptidoglycan complex of Mycobacterium species, the protein peptidoglycan complex and muramyl dipeptide all trigger release of tumour necrosis factor (TNF).

Claims 1, 2, 5-10, 15-20 and 22 lack both novelty and inventive step when compared with the disclosure in Barnes et al (1992).

WO 96/00579 (Document (o)) discloses aqueous cell wall fractions isolated from Mycobacterium. The isolates have been used to treat infections, neoplastic disorders, bronchial asthma, allergies of unknown sources and rheumatoid arthritis. Treatment of human patients with diabetes is not disclosed.

Claims 1-3, 5, 20 and 22 lack both novelty and inventive step when compared with the disclosure in WO 96/00579.

WO 96/26288 (Document (p)) discloses a method of separation of a specific microbial cell wall component to obtain mycolic acid from species of Mycobacterium. The method disclosed anticipates the method claimed in claims 17-19.

WO 94/16727 (Document (q)) discloses a modified Mycobacterial cell wall extract, which is of use in stimulating the immune system of a mammal. Claims 1, 2, 10 and 20 lack both novelty and inventive step when compared with WO 94/16727.

The Applicants argument that "none of the documents cited by the authority disclose or even allude to the use of components of mycobacterium in immunomodulatory therapy" are not found to be persuasive.All of the documents cited disclose the use of mycobacterial extracts in methods of treatment which act via immunomodulation for example in vaccines. Mycobacterial extracts are well known adjuvants used in vaccines.

Claims 1-10, 17-22 are directed to a method of immunomodulation. A number of publications listed above disclose aspects of observed immunomodulation following administration of Mycobacterial extracts, including immunological responses to vaccination with Mycobacterial extracts.

Please Note:

Under Rule 67.1of the PCT claims, which have as the subject matter a Method of Treatment, are considered excluded subject matter and do not require an International Preliminary Examination. However, because the subject matter of the Method of Treatment claims is allowable under Australian law the Method of Treatment claims have been examined in this International Preliminary Examination Report.

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

The claims are speculative and not fairly based on the disclosure of the specification using the examples. The claims include cell wall fractions, which fall outside the scope of the invention. For example deproteinized cell wall fractions which are known fall within the scope of the claims. The applicant has found that MAPG is the active fraction. Earlier work has been published (such as the publications cited in the International Search Report) on the activities of cell wall fractions of mycobacteria. Earlier workers have found different activities for different cell wall fractions.

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 1958617/EJH/AF	**FOR FURTHER ACTION**	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416).
International application No. **PCT/AU 97/00770**	International filing date *(day/month/year)* 13 November 1997	Priority Date *(day/month/year)* 13 November 1996
International Patent Classification (IPC) or national classification and IPC **Int. Cl.**[6] A61K 39/04, A61K 38/02; C07K 14/35		
Applicant AMRAD OPERATIONS PTY LTD et al		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of **8** sheets, including this cover sheet.

☐ This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

These annexes consist of a total of sheet(s).

3. This report contains indications relating to the following items:

I	☒	Basis of the report
II	☐	Priority
III	☐	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
IV	☐	Lack of unity of invention
V	☒	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
VI	☐	Certain documents cited
VII	☐	Certain defects in the international application
VIII	☒	Certain observations on the international application

Date of submission of the demand 12 June 1998	Date of completion of the report 8 March 1999
Name and mailing address of the IPEA/AU AUSTRALIAN PATENT OFFICE PO BOX 200 WODEN ACT 2606 AUSTRALIA Facsimile No. (02) 6285 3929	Authorized Officer **ARATI SARDANA** Telephone No. (02) 6283 2627

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/AU 97/00770

I. Basis of the report

1. With regard to the **elements** of the international application:*

[X] the international application as originally filed.

[ ] the description, pages , as originally filed,
pages , filed with the demand,
pages , filed with the letter of .

[ ] the claims, pages , as originally filed,
pages , as amended (together with any statement) under Article 19,
pages , filed with the demand,
pages , filed with the letter of .

[ ] the drawings, pages , as originally filed,
pages , filed with the demand,
pages , filed with the letter of .

[ ] the sequence listing part of the description:
pages , as originally filed
pages , filed with the demand
pages , filed with the letter of

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.
These elements were available or furnished to this Authority in the following language which is:

[ ] the language of a translation furnished for the purposes of international search (under Rule 23.1(b)).

[ ] the language of publication of the international application (under Rule 48.3(b)).

[ ] the language of the translation furnished for the purposes of international preliminary examination (under Rules 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, was on the basis of the sequence listing:

[ ] contained in the international application in written form.

[ ] filed together with the international application in computer readable form.

[ ] furnished subsequently to this Authority in written form.

[ ] furnished subsequently to this Authority in computer readable form.

[ ] The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

[ ] The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

4. [ ] The amendments have resulted in the cancellation of:

[ ] the description, pages

[ ] the claims, Nos.

[ ] the drawings, sheets/fig

5. [ ] This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).**

* *Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17).*

** *Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report*

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims **4 and 11-14**	**YES**
	Claims **1-3, 5-10 and 15-22**	**NO**
Inventive step (IS)	Claims **4 and 11-14**	**YES**
	Claims **1-3, 5-10 and 15-22**	**NO**
Industrial applicability (IA)	Claims **1-22**	**YES**
	Claims	**NO**

2. Citations and explanations (Rule 70.7)

Citations

a) WO 87/02249 A (Ragland, William L) 23 April 1987
entire document

b) GB 2120548 A (Ribi Immunochem Research Inc) 7 December 1983
entire document

c) US 4152423 A (Adam et al) 1 May 1979
entire document

d) Hirschfield, G R *et al* "Peptidoglycan-Associated Polypeptides of *Mycobacterium tuberculosis*" J Bacteriol (February 1990) 172(2), 1005-1013
abstract

e) Azuma, I *et al*, "Fractionation of Mycobacterial Cell Wall" *J Bacteriol* (November 1968) 96(5), 1885-1887
entire document

f) Baxter, A G *et al*, "Mycobacteria precipitate an SLE-like syndrome in diabetes-prone NOD mice", *Immunology*
abstract and discussion

g) Sadelain, M W J *et al*, "Prevention Type I diabetes in NOD mice by adjuvant immunotherapy", *Diabetes* (May 1990), 39(5), 583-587
abstract

h) Harada, M *et al*, "Prevention of overt diabetes and insulitis in NOD mice by a single BCG vaccination" *Diabetes Res Clin Prac* (1990) 8, 85-89
abstract

Supplemental Box

(To be used when the space in any of the preceding boxes is not sufficient)

Continuation of : V

i) Mediline Abstract UI: 93171635, Qin, M y *et al*, "Complete Freund's adjuvant-induced T-cells prevent the development and adaptive transfer of diabetes in non-obese diabetic mice", *J Immunol* (1993), 150(5), 2072-2080
abstract

j) Mediline Abstract UI: 93209598, Chugh, I B *et al*, "Protective efficacy of different cell-wall fractions of Mycobacterium tuberculosis",*Folia, Microbiol (Praha)*, (1992), 37(6), 407-412
abstract

k) Mediline Abstract UI: 83254632, Beaudet, R *et al*, "Stimulation of non-specific anti-tumor resistance in the mouse using cell wall preparations from four BCG substrains", *Ann Immunol (Paris)* (1983), 134C(2), 215-226
abstract

l) Dockrell, H M *et al*, "Induction of Thl Cytokine Responses by Mycobacterial Antigens in Leprosy", *Infect Immun*, (October 1996), 64(10, 4385-4389)
abstract

m) McNeil, M *et al*, "Evidence for the Nature of the Link between the Arabinogalactan and Peptidoglycan of Mycobacterial Cell Walls" *J Biol Chem* (25 October 1990) 265(30), 18200-18206

n) Barnes, PF *et al*, "Tumour Necrosis Factor Production in Patients with Leprosy", *Infect Immun.* (April 1992), 60(4), 1441-1446
abstract

o) WO 96/00579 A (Lavesarznimittel GmbH) 11 January 1996
entire document

p) WO 96/26288 A (Adcock Ingram Limited) 29 August 1996
entire document

q) WO 94/16727 A (Vetrepharm, Inc) 4 August 1994
entire document

Explanations

The claims of PCT/AU97/00770 are directed to the use of components of the cell wall of Mycobacterium in methods of treatment for autoimmune disease including insulin-dependant diabetes mellitis (IDDM), anti-tumour immune response, melanoma and bladder cancer. Claims 15 and 16 are directed to a composition of matter comprising mycolyarabinogalactan-pepidoglycon (MAPG). Claims 17-19 are directed to methods of isolation of MAPG. Claims 1-14 and 20-22 are directed to methods of immunomodulatory therapy of a mammal using one or more of the components of the cell wall of Mycobacterium which includes MAPG.

WO 87/02249 (Document (a)) describes and claims an antiviral immunotherapeutic agent which comprises an effective amount of deproteinized bacterial cell wall suspension. Claim 2 is directed to bacteria selected from the group consisting of Mycobacterium species. Claim 11 describes a method of isolation of cell walls of bacteria and the preparation of an antiviral immunotherapeutic agent. Example 10 discloses the treatment of herpesvirus infection in horses known as acute rhinopneumonitis, using Mycobacterium cell wall extract.

Supplemental Box
(To be used when the space in any of the preceding boxes is not sufficient)

Continuation of: V

Claims 1-3, 5, 6, 10 and 17-20 lack both novelty and inventive step when compared with the disclosure and claims of WO 87/02249 (Document (a)).

GB 2120548 (Document (b)) discloses and claims a method of isolation of cell wall skeleton from mycobacteria including Mycobacterium bovis. The applicant has isolated a composition containing cell wall skeleton (CWS) and purified trehalose dimycolates (TDM). The composition has been shown to be efficient in obtaining suppression and regression of tumour cells.

Claims 1, 2, 5-10 and 17-22 are not novel and lack an inventive step when compared with the disclosure and claims of GB 2120548 (Document (b)).

US 4152423 (Document (c)) discloses the use of cell wall extracts of Mycobacterium to produce vaccine preparations against bacterial, viral and parasitic infections and antigens for tumours (see column 14 lines 60-68). Materials included in the scope of the present invention are well known for use as adjuvants to increase the efficiency of vaccines, especially if they are weak immunogens. The monomer units of the wall include a mucopeptide combined with a glycolipid containing an arabinogalactan.

Claims 1-3, 5, 7, 8, 10 and 15-20 are not novel and lack an inventive step when compared with US 4152423 (Document (c)).

Hirschfield et al, (1990) (Document (d)) discloses cell wall polypeptides isolated from Mycobacterium. Purified cell walls of Mycobacterium tuberculosis were extracted with sodium dodecyl sulphate to produce an insluble residue composed of the mycolylarabinogalactan-peptidoglycan complex. The publication does not teach the possible uses of the complex in a method of immunomodulatory therapy. However, a person skilled in the art would read the disclosure by Hirschfield et al, (1990) in the light of common general knowledge and find that a claim to the method of immunomodulation would lack an inventive step. Claims 1, 2 and 20 lack an inventive step and claims 15-19 lack novelty when compared with the disclosure of Hirschfield et al. (Document(d)).

Azuma et al, (1968) (Document (e)) teaches the isolation of cell wall components of Mycobacterium tuberculosis. At page 1886, column 2, at the last paragraph work by Kanetsuna is cited which suggests that mycolic acid-arabinoglactan-mucopeptide complex may be a common structure of the mycobacterial cell wall. The publication does not teach a method of treatment. Claims 15-19 lack novelty when compared with the disclosure by Azuma et al.

Baxter et al, (1994) (Document (f)) discloses the use of preparations from Mycobacterium bovis to prevent the development of diabetes in non-obese diabetic mice. There is no disclosure that cell wall components are of use as vaccines. The disclosure does not deprive any of the claims of novelty or inventive step. A similar comment applies to the publication by Sadelain et al, (1997), Harada et al, (1990) and Qin et al, (1993). Claims 1-22 are both novel and have an inventive step when compared with these publications.

Sadelain, MWJ, et al (Document (g)) discloses the treatment of diabetes in non-obese diabetic (NOD) mice. NOD mice were given a single injection of complete Freund's adjuvant. Animals receiving Freund's adjuvant showed microscopic evidence of healthy insulin-secreting cells for up to eighteen months. Evaluation of immune system function in the treated and untreated animals indicate that CFA caused an apparently permanent decrease in the level of T-lymphocytes, a type of cell associated with the progression of diabetes. The results suggest possible treatment for diabetes as well as for other autoimmune disorders.

Supplemental Box
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Continuation of: V

A single injection of Freund's adjuvant (CFA) given at early age prevented the appearance of diabetes and greatly increased the life span of NOD mice without additional therapy. The non-obese diabetic (NOD) mice spontaneously develop an insulin-dependent diabetes mellitis that has many immunological and pathological similarities to human insulin-dependent diabetes. Freund's adjuvant comprises killed Mycobacterium.

Claims 1-4, 10-13, 20 and 21 lack both novelty and inventive step when compared with the disclosure in Document (g).

Harada, M et al, (1990) (Document (h)) discloses that a single intravenous injection of live Bacillus Calmette-Guèrin BCG at ten weeks of age produced a potent suppression of insulitis and overt diabetes in non-obese, diabetes-prone (NOD) female mice(see the abstract). Earlier work disclosing the use of live bacillus Calmette- Guèrin (BCG) against cancers and Leishmania infection (page 85, column 2). Bacillus Calmette- Guèrin (BCG) is also known as Mycobacterium Tuberculosis.

Claims 1-6, 11-13 and 20-22 lack both novelty and inventive step when compared with the disclosure in Document (h).

Qin, HY, et al, (1993) (Document (i)) discloses that mice injected with Mycobacterium tuberculosis or Mycobacterium bovis (BCG) vaccine at four weeks of age were found to be protected from diabetes. Claims 1-4, 11-14, 20 and 21 lack both novelty and inventive step when compared with Document (i).

Chugh et al, (1992) (Document (j)) discloses immunisation with cell wall fractions from Mycobacteria of mycolic acids and arabinogalactans (cell-wall-protein-peptidoglycan complex). Mice immunised with cell wall fractions had increased protection when challenged with Mycobacterium tuberculosis. Claims 1, 2 and 20 lack novelty and inventive step when compared with the disclosure by Chugh et al, (1992).

Beaudet et al, (1983) (Document (k)) describes a study of the anti-tumour activity of cell walls, deproteinized cell walls and cell wall skeleton isolated from four BCG (Mycobacterium) substrains. The preparations were found to offer significant protection against Ehrlich carcinoma but were no protection for L1210 leukaemia. There is no disclosure of a method of treatment of insulin-dependent diabetes mellitis (IDDM) or of isolation of mycolyl-arabinogalactan-peptidoglycan (MAPG). Claims 1, 2, 5, 6, 20 and 22 lack both novelty and inventive step when compared with the publication by Beaudet et al, (1983).

Dockrell et al, (1996) (Document (l)) used mycobacterial antigens including mycolylarabinogalactan peptidoglycan to produce an interferon gamma response in tuberculoid leprosy. A method of isolation of mycolylarabino-galactan peptidoglycan is disclosed. Claims 1, 2, 7, 15, 16 and 20 lack novelty and inventive step when compared with the disclosure by Dockerell et al, (1996).

McNeil et al, (1990) (Document (m)) discloses the isolation and analysis of mycolylarabinogalactan and the peptidoglycan of the cell walls of Mycobacterium bovis, Mycobacterium leprae and Mycobacterium tuberculosis. There is no disclosure of the use of cell wall components in method of treatment. Claims 15-19 lack both novelty and inventive step when compared to McNeil et al.

Barnes et al (1992) studied the effects of mycobacterium on the role of tumour necrosis factor (TNF) in leprosy. Claims 1, 2, 7, 8, 10 and 15-20 lack both novelty and inventive step when compared with Barnes et al, (1992).

Supplemental Box
(To be used when the space in any of the preceding boxes is not sufficient)

Continuation of:V

Barnes, PF et al (1992) (Document (n)) discloses the host response to Mycobacterium leprae. Treatment of human patients with Mycobacterium leprae, mycolylarabinogalactan peptidoglycan complex of Mycobacterium species, the protein peptidoglycan complex and muramyl dipeptide all trigger release of tumour necrosis factor (TNF).

Claims 1, 2, 5-10, 15-20 and 22 lack both novelty and inventive step when compared with the disclosure in Barnes et al (1992).

WO 96/00579 (Document (o)) discloses aqueous cell wall fractions isolated from Mycobacterium. The isolates have been used to treat infections, neoplastic disorders, bronchial asthma, allergies of unknown sources and rheumatoid arthritis. Treatment of human patients with diabetes is not disclosed.

Claims 1-3, 5, 20 and 22 lack both novelty and inventive step when compared with the disclosure in WO 96/00579.

WO 96/26288 (Document (p)) discloses a method of separation of a specific microbial cell wall component to obtain mycolic acid from species of Mycobacterium. The method disclosed anticipates the method claimed in claims 17-19.

WO 94/16727 (Document (q)) discloses a modified Mycobacterial cell wall extract, which is of use in stimulating the immune system of a mammal. Claims 1, 2, 10 and 20 lack both novelty and inventive step when compared with WO 94/16727.

The Applicants argument that "none of the documents cited by the authority disclose or even allude to the use of components of mycobacterium in immunomodulatory therapy" are not found to be persuasive.All of the documents cited disclose the use of mycobacterial extracts in methods of treatment which act via immunomodulation for example in vaccines. Mycobacterial extracts are well known adjuvants used in vaccines.

Claims 1-10, 17-22 are directed to a method of immunomodulation. A number of publications listed above disclose aspects of observed immunomodulation following administration of Mycobacterial extracts, including immunological responses to vaccination with Mycobacterial extracts.

Please Note:

Under Rule 67.1of the PCT claims, which have as the subject matter a Method of Treatment, are considered excluded subject matter and do not require an International Preliminary Examination. However, because the subject matter of the Method of Treatment claims is allowable under Australian law the Method of Treatment claims have been examined in this International Preliminary Examination Report.

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

The claims are speculative and not fairly based on the disclosure of the specification using the examples. The claims include cell wall fractions, which fall outside the scope of the invention. For example deproteinized cell wall fractions which are known fall within the scope of the claims. The applicant has found that MAPG is the active fraction. Earlier work has been published (such as the publications cited in the International Search Report) on the activities of cell wall fractions of mycobacteria. Earlier workers have found different activities for different cell wall fractions.

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(54) Title: MYCOBACTERIUM CELL WALL COMPOSITIONS

(57) Abstract

The present invention relates generally to a method of immunomodulating therapy and pharmaceutical compositions useful for same. More particularly, the present invention provides a method of ameliorating the effects of autoimmune conditions. Even more particularly, the present invention contemplates a method for preventing, delaying onset of or otherwise ameliorating the effects of insulin–dependant diabetes mellitus (IDDM) by administering a cell wall subunit or a chemical or functional equivalent thereof from *Mycobacterium* or a related organism or other suitable biological source. The present invention is further directed to a pharmaceutical composition useful in preventing, delaying onset of, curing, curing in association with islet replacement or otherwise ameliorating the effects of autoimmune conditions such as IDDM or for enhancing an immune response against melanoma or other cancer comprising a cell wall subunit or a chemical or functional equivalent thereof from *Mycobacterium* or a related organism or other suitable biological source. The cell wall subunit is preferably mycolyl–arabinogalactan–peptidoglycan (MAPG) or a component thereof.

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MYCOBACTERIUM CELL WALL COMPOSITIONS

FIELD OF THE INVENTION

The present invention relates generally to a method of immunomodulating therapy and pharmaceutical compositions useful for same. More particularly, the present invention provides a method of ameliorating the effects of autoimmune conditions. Even more particularly, the present invention contemplates a method for preventing, delaying onset of or otherwise ameliorating the effects of insulin-dependent diabetes mellitus (IDDM) by administering a cell wall subunit or a chemical or functional equivalent thereof from *Mycobacterium* or a related organism or other suitable biological source. The present invention is further directed to a pharmaceutical composition useful in preventing, delaying onset of, curing, curing in association with islet replacement or otherwise ameliorating the effects of autoimmune conditions such as IDDM or for enhancing an immune response against melanoma or other cancer comprising a cell wall subunit or a chemical or functional equivalent thereof from *Mycobacterium* or a related organism or other suitable biological source.

Throughout this specification and the claims which follow, unless the context requires otherwise, the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated integer or group of integers but not the exclusion of any other integer or group of integers.

Bibliographic details of the publications numerically referred to in this specification are collected at the end of the description.

BACKGROUND OF THE INVENTION

Insulin-dependent diabetes mellitis (IDDM) is a debilitating, chronic, cell-mediated autoimmune disease characterised by lymphocytic infiltration of the pancreatic islets and T lymphocyte-

mediated destruction of insulin-producing β cells (1, 2).

Non-obese diabetic (NOD) mice are a valuable model in studying IDDM as they spontaneously develop the disease which has many immunological and pathological similarities to human IDDM (3, 4).

It has been previously shown that administration of Freund's complete adjuvant (CFA) or *Mycobacterium bovis* (Bacillus Calmette-Guerin (BCG) [3]) prevents development of diabetes in NOD mice (5, 6). However, Baxter *et al* (7) showed the administration of BCG, although preventing diabetes in NOD mice, precipitated a syndrome similar to systemic lupus erythematosus (SLE), precluding its use in humans.

In accordance with the present invention, it has been shown that a subunit complex from the cell wall of *Mycobacterium* prevents diabetes in NOD mice without risk of precipitating SLE. The subunit complex , or one or more of its components, are useful, therefore in immunomodulatory therapy for autoimmune diseases and for enhancing an immune response to various cancers.

SUMMARY OF THE INVENTION

One aspect of the present invention contemplates a method of immunomodulatory therapy in a mammal said method comprising administering to said mammal an immunomodulating effective amount of one or more components of the cell wall of *Mycobacterium* or a related organism or analogous components from another biological source.

Another aspect of the present invention provides a method of preventing, delaying onset of, curing or otherwise ameliorating the effects of an autoimmune disease in a mammal said method comprising administering to said mammal an autoimmune-preventing effective amount of one or more components of the cell wall of *Mycobacterium* or a related organism or analogous components from another biological source.

Still another aspect of the present invention is directed to a method of preventing, delaying onset

of, curing or otherwise ameliorating the effects of insulin-dependent diabetes mellitus (IDDM) in a mammal said method comprising administering to said mammal an autoimmune-preventing effective amount of one or more components of the cell wall of *Mycobacterium* or a related organism or analogous components from another biological source.

Yet another aspect of the present invention contemplates a method of enhancing an immune response against melanoma or other cancer in a mammal said method comprising administering to said mammal an immunomodulatory effective amount of one or more components of the cell wall of *Mycobacterium* or a related organism or analogous components from another biological source.

Still yet another aspect of the present invention contemplates immunomodulatory therapy in a mammal said method comprising administering to said mammal an immunomodulatory effective amount of mycolyl-arabinogalactan-peptidoglycan (MAPG) or a component thereof with or without other associated cell wall components and submolecular components from a *Mycobacterium* species such as but not limited to *Mycobacterium bovis* or a chemical equivalent of said MAPG or of a component thereof.

Even yet another aspect of the present invention contemplates a method for isolating components of MAPG for use in a therapeutic composition for preventing, delaying the onset of or otherwise ameliorating the effects of diabetes in a mammal or for use in immunomodulatory therapy said method comprising preparing cell envelopes from a species of *Mycobacterium* or related organism or other suitable biological source, subjecting said cell envelopes to glycolipid removing means to remove soluble glycolipids, treating the product so obtained to break the mycolic acids linkage and isolating said mycolic acids, treating the remaining complex to cleave linkage at rhamnose residue connecting arabinogalactan to the peptidoglycan backbone and separating and isolating arabinogalactan and peptidoglycan.

Another aspect of the present invention provides a composition of matter comprising MAPG or a derivative or a component thereof or its derivative.

Yet another aspect of the present invention relates to the use of a cell wall component of *Mycobacterium* in immunomodulatory therapy.

BRIEF DESCRIPTION OF THE FIGURES

In the Figures:

Figure 1 is a diagrammatic representation of a mycobacterial cell wall.

Figure 2 is a graphical representation showing the percentage incidence of diabetes over time (days) following administration of phosphate buffered saline (PBS).

Figure 3 is a graphical representation showing the percentage incidence of diabetes over time (days) following administration of BCG.

□ 0.1 mg/mouse

+ 0.2 mg/mouse

○ 0.4 mg/mouse

◇ 0.8, 1.6 , 4 mg/mouse

Figure 4 is a graphical representation showing the percentage incidence of diabetes over time (days) following administration of N-CFAglucosaminyl-N-acetylmuramyl-L-alanyl-D-isoglutamine I.V. (1.0-10μg).

□ 1.0 μg/mouse

+ 2.5 μg/mouse

○ 5.0 μg/mouse

◇ 10 μg/mouse

Figure 5 is a graphical representation showing the percentage incidence of diabetes over time (days) following administration of lipoarabinomannan (6.25-50μg I.V.).

□ 6.25 μg/mouse

+ 12.5 μg/mouse

○ 25 μg/mouse

◇ 50 μg/mouse

Figure 6 is a graphical representation showing the percentage incidence of diabetes over time (days) following administration of mycolyl-arabinogalactan-peptidoglycan (0.125-1.0 mg I.V.).

□ 0.125 mg/mouse

+ 0.25 mg/mouse

○ 0.5 mg/mouse

◇ 1.0 mg/mouse

Figure 7 is a graphical representation showing the percentage incidence of diabetes over time (days) following administration of mycolic acids (13-200 μg I.V.).

□ 12.5 μg/mouse

+ 50 μg/mouse

○ 200 μg/mouse

DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

The present invention contemplates a method of immunomodulatory therapy in a mammal said method comprising administering to said mammal an immunomodulating effective amount of one or more components of the cell wall of *Mycobacterium* or a related organism or analogous components from another biological source or chemical equivalents of said components.

In one aspect, the present invention is directed to a method of preventing, delaying onset of, curing or otherwise ameliorating the effects of an autoimmune disease in a mammal said method comprising administering to said mammal an autoimmune-preventing effective amount of one or more components of the cell wall of *Mycobacterium* or a related organism or analogous components from another biological source or chemical equivalents of said components.

In another aspect, the present invention provides a method of enhancing an immune response against melanoma or other cancer in a mammal said method comprising administering to said mammal an immunomodulatory effective amount of one or more components of the cell wall of *Mycobacterium* or a related organism or analogous components from another biological source or chemical equivalents of said components.

Autoimmune conditions contemplated by the present invention include but are not limited to IDDM, thyroiditis, atrophic gastritis (type A), pernicious anaemia, Addison's disease, pemphigus vulgaris, pemphigoid, multiple sclerosis, rheumatoid arthritis, systemic lupus erythematosus, discoid lupus erythematosus, haemolytic anaemia, sympathetic ophthalmia, uveitis, idiopathic thrombocytopenia, idiopathic leucopenia, primary biliary cirrhosis, autoimmune chronic active hepatitis, ulcerative colitis, Sjogren's syndrome, dermatomyositis, scleroderma and mixed connective tissue disease.

Cancers contemplated for immunomodulatory therapy include but are not limited to bladder cancer, carcinoma, melanoma amongst many others.

The present invention is hereinafter described in relation to IDDM but this is done with the

understanding that the invention extends to autoimmune diseases such as contemplated above as well as the immunomodulatory therapy of cancers.

Accordingly, the present invention particularly contemplates a method of preventing, delaying onset of, curing, curing in association with islet replacement and/or pancreas transplant or otherwise ameliorating the effects of IDDM in a mammal said method comprising administering to said mammal an IDDM-preventing effective amount of one or more components of the cell wall of *Mycobacterium* or a related organism or analogous components from another biological source or chemical equivalents of said components.

The major components of the mycobacterial cell wall are: 1) mycolyl-arabinogalactan-peptidoglycan (MAPG), a polymer which provides the structural framework of the cell wall, 2) lipids, 3) phosphatidylinositol mannosides, and 4) lipoarabinomannan (LAM). MAPG has three major subdomains which are: 1) peptidoglycan, 2) arabinogalactan and 3) mycolic acids. N-acetylglucosaminyl-N-acetylmuramyl-L-alanyl-D-isoglutamine (GMDP) and N-acetylmuramyl-L-alanyl-D-isoglutamine (MDP) are peptidoglycan subunits considered to be responsible for most of the adjuvant activity of CFA.

The components of the *Mycobacterium* cell wall or of another suitable source or their chemical equivalents contemplated for use in preventing IDDM in mammals include MAPG or components or chemical equivalents thereof with or without other associated cell wall components and submolecular components or such as but not limited to mycolate, arabinogalactan and/or peptidoglycan or derivatives or chemical equivalents thereof (see Figure 1). MAPG or its components may be in native or chemically synthetic form. MAPG is a complex of covalently attached macromolecules. Mycolic acids are covalently attached to arabinogalactan which is in turn covalently attached to peptidoglycan. Reference herein to "MAPG" includes the MAPG complex isolated from *Mycobacterium* or related organism or other suitable biological source or to a chemically or functionally equivalent complex as well as submolecular components including mycolic acids, peptidoglycan or arabinogalactan or chemical or functional equivalents thereof. The submolecular components may be in isolated form or in partial complex forms such as comprising mycolic acids and arabinogalactan, arabinogalactan and

peptidoglycan or mycolic acids and peptidoglycan or chemical or functional equivalents thereof. A particular complex may also comprise, for example, mycolic acids covalently linking to arabinogalactan and this may in turn be covalently linked to a portion or derivative of peptidoglycan.

A convenient source of MAPG or its components is *Mycobacterium bovis* or BCG. The present invention, however, extends to MAPG or its components from any species of *Mycobacterium* or from physiologically, genetically, biochemically or structurally related microorganisms. Examples of similar organisms include *Actinomycetes*, *Nocardia* and *Corynebacterium*. A similar molecule or natural complex or components thereof may also be isolatable for other biological sources such as plants and coral. The MAPG complex or its components may be isolated from mycobacterial cell envelopes prepared, for example, by the method of Azuma *et al* (8)

According to this aspect of the present invention, there is provided a method for isolating components of MAPG for use in a therapeutic composition for preventing, delaying the onset of or otherwise ameliorating the effects of diabetes in a mammal, said method comprising preparing cell envelopes from a species of *Mycobacterium* or related organism or other suitable biological source, subjecting said cell envelopes to glycolipid removing means to remove soluble glycolipids, treating the product so obtained to break the mycolic acids linkage and isolating said mycolic acids, treating the remaining complex to cleave linkage at rhamnose residue connecting arabinogalactan to the peptidoglycan backbone and separating and isolating arabinogalactan and peptidoglycan.

The soluble glycolipids are conveniently removed by repeated centrifugation in the presence of sodium dodecyl sulphite (SDS). The resulting insolvent envelope MAPG complex is then subjected to fractionation.

The mycolic acids linkage is preferably cleaved by saponification, base-catalysed methanolysis or ammonolysis. The remaining complex of arabinogalactan and peptidoglycan is preferably then subjected to a Smith degradation comprising periodate followed by borohydride reduction and

mild acid treatment.

The MAPG complex or its component parts or derivatives thereof may be in any convenient form such as vacuum dried, powder, liquid or slurry.

The present invention further contemplates a composition of matter comprising MAPG or a derivative thereof or a component thereof or its derivative or chemical equivalents of MAPG or its components. These components are referred to herein as "active ingredients".

Preferably, the composition is a pharmaceutical composition for use in preventing, delaying onset of, curing, curing in association with islet replacement or otherwise ameliorating the effects of IDDM in mammals or for enhancing an anti-tumour immune response in mammals. The pharmaceutical composition may additionally comprise one or more pharmaceutically acceptable carriers and/or diluents.

According to this and other aspects of the present invention, preferred mammals include humans, primates, livestock animals (eg. cows, horses, sheep, pigs, donkeys), laboratory test animals (eg. mice, rabbits, guinea pigs, hamsters), companion animals (eg. dogs, cats) and captive wild animals (eg. kangaroos, foxes, deer).

The pharmaceutical forms suitable for injectable use include sterile aqueous solutions (where water soluble) and sterile powders for the extemporaneous preparation of sterile injectable solutions. The present invention also contemplates administration *via* topically applied compositions where molecules are used to permit entry *via* the skin. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi.

The carrier may be a solvent medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol and liquid polyethylene glycol and the like) or suitable mixtures thereof and vegetable oils. The preventions of the action of microorganisms can be brought about by various antibacterial and antifungal agents, for example, parabens,

chlorobutanol, phenol, sorbic acid, thirmerosal and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars or sodium chloride. Prolonged absorption of the injectable compositions can be brought about by the use in the compositions of agents delaying absorption, for example, aluminum monostearate and gelatin.

Sterile injectable solutions are prepared by incorporating the active compounds in the required amount in the appropriate solvent with various of the other ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the various sterilized active ingredients into a sterile vehicle which contains the basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and the freeze-drying techniques which yield a powder of the active ingredient plus any additional desired ingredient from previously sterile-filtered solution thereof.

When the active ingredients are suitably protected they may be orally administered, for example, with an inert diluent or with an assimilable edible carrier, or it may be enclosed in hard or soft shell gelatin capsule, or it may be compressed into tablets, or it may be incorporated directly with food material (including solid or liquid products). For oral therapeutic administration, the active compound may be incorporated with excipients and used in the form of ingestible tablets, buccal tablets, troches, capsules, elixirs, suspensions, syrups, wafers, and the like. Such compositions and preparations should contain at least 1% by weight of active compound. The percentage of the compositions and preparations may, of course, be varied and may conveniently be between about 5 to about 80% of the weight of the unit. The amount of active compound in such therapeutically useful compositions is such that a suitable dosage will be obtained. Preferred compositions or preparations according to the present invention are prepared so that an oral dosage unit form contains between about 0.1 μg and about 2000 mg of active compound. Other ranges contemplated herein include from about 1μg to about 1000 mg, from about 10 μg to about 100 mg and from about 100 μg to about 50 mg. Effective amounts may also be provided as an amount per kilogram of body weight of the recipient. For example, from about 0.01 ng to about 10,000 mg/kg body weight or may be administered from about 0.1 ng to about 500 mg/kg body weight.

The tablets, troches, pills, capsules and the like may also contain the components as listed hereafter: a binder such as gum, acacia, corn starch or gelatin; excipients such as dicalcium phosphate; a disintegrating agent such as corn starch, potato starch, alginic acid and the like; a lubricant such as magnesium stearate; and a sweetening agent such a sucrose, lactose or saccharin may be added or a flavouring agent such as peppermint, oil of wintergreen, or cherry flavouring. When the dosage unit form is a capsule, it may contain, in addition to materials of the above type, a liquid carrier. Various other materials may be present as coatings or to otherwise modify the physical form of the dosage unit. For instance, tablets, pills, or capsules may be coated with shellac, sugar or both. A syrup or elixir may contain the active compound, sucrose as a sweetening agent, methyl and propylparabens as preservatives, a dye and flavouring such as cherry or orange flavour. Of course, any material used in preparing any dosage unit form should be pharmaceutically pure and substantially non-toxic in the amounts employed. In addition, the active compound(s) may be incorporated into sustained-release preparations and formulations.

The present invention also extends to forms suitable for topical application such as creams, lotions and gels.

Pharmaceutically acceptable carriers and/or diluents include any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents and the like. The use of such media and agents for pharmaceutical active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active ingredient, use thereof in the therapeutic compositions is contemplated. Supplementary active ingredients can also be incorporated into the compositions.

It is especially advantageous to formulate parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Parental compositions may be administered by, for example, intravenous (IV), subcutaneous (SC) or intramuscular (IM) routes amongst other routes. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the mammalian subjects to be treated; each unit containing a predetermined quantity of active material calculated to produce the desired therapeutic effect in association with the

required pharmaceutical carrier. The specification for the novel dosage unit forms of the invention are dictated by and directly dependent on (a) the unique characteristics of the active material and the particular therapeutic effect to be achieved, and (b) the limitations inherent in the art of compounding such an active material for the treatment of disease in living subjects having a diseased condition in which bodily health is impaired as herein disclosed in detail. Other routes of administration are also contemplated by the present invention including intracerebral, intraperitoneal, intranasal, buccal, rectal, implant, infusion, inhalation administration in addition to intravenous drip.

The principal active ingredient is compounded for convenient and effective administration in effective amounts with a suitable pharmaceutically acceptable carrier in dosage unit form as hereinbefore disclosed. A unit dosage form can, for example, contain the principal active compound in amounts ranging from about 0.1 μg to about 2000 mg. Expressed in proportions, the active compound is generally present in from about 0.5 μg to about 2000 mg/ml of carrier. In the case of compositions containing supplementary active ingredients, the dosages are determined by reference to the usual dose and manner of administration of the said ingredients.

The active ingredients of the present invention may be administered alone or in combination with other therapeutic molecules such as molecules which reduce effects of the autoimmune pathology associated with IDDM. Alternatively, the active ingredients may be administered with anti-cancer agents or functionally unrelated but nevertheless useful molecules such as antibodies, analogues or the like. A single dose may be administered or multiple doses may be required with intervals of from minutes to hours, daily to weekly or monthly to yearly.

Reference herein to "preventing" IDDM includes total prevention of IDDM or substantial prevention for a limited time (eg. from about 1 to about 10 years) or delaying onset of IDDM or reducing the severity or otherwise ameliorating the effects of IDDM.

The present invention further contemplates use of a cell wall component of *Mycobacterium* in immunomodulatory therapy. Preferably, the therapy is the treatment of an autoimmune disease such as diabetes. Alternatively, the therapy is the treatment of melanoma, bladder cancer or

other cancer, preferably by enhancing an immune response.

The present invention is further described by the following non-limiting Examples.

EXAMPLE 1

Components of mycobacterial cell wall

Components of the mycobacterial cell wall are shown in Figure 1.

EXAMPLE 2

Mice

Female NOD/Lt//Arc mice were obtained from the Animal Resources Centre (Canning Vale, WA, Australia) and maintained in clean conditions in the Centenary Institute Animal House. Sentinel mice were tested by serology at four-monthly intervals for the following pathogens: mouse hepatitis virus, rotavirus, ectomclia, mouse cytomegalovirus, polyoma virus, murine adenovirus, lymphocytic choriomeningitis virus, mouse pneumonia virus, retrovirus, Sendai virus, Theiler's murine encephalitis virus, *Bacillus piliformis, Mycoplasma pulmonis, Bordetella bronchiseptica, Corynebacterium kutscheri, Klebsiella* species, *Pasturella multocide, Pasturella pneumotropica, Pseudomonas aeruginosa, Straphylococcus aureus, Streptococcus pneumoniae, Citrobacter freundii* and salmonella species. No mice tested positive for any of these pathogens. Mice were housed at 21C and 40% humidity and were fed Barastock mouse chow (Melbourne, VIC, Australia) and acidified water *ad libitum.*

Under these conditions, about 75% of female NOD mice spontaneously developed IDDM by 35 weeks of age. The disease process involved a progressive preclinical phase of islet destruction which commenced at 4-6 weeks of age, and concluded with the onset of clinical diabetes between 14 and 35 weeks of age. Within a population, disease onset occurred in a sigmoidal fashion with the peak incidence of IDDM at 22 weeks of age, and a plateau at 35 weeks after which few previously unaffected mice ever progressed to diabetes (9).

NOD mice intravenously injected with a single dose of 1.0-4.0mg of heat-killed BCG did not become diabetic, but developed a lupus-like disease characterised by haemolytic anaemia (indicated by a lowered haematocrit and positive Coombs' test), increased titres of anti-nuclear antibodies (demonstrated by immunofluorescence of HEp-2 cells) and glomerulonephitis (demonstrated by immunofluorescence of C3c bound to the renal glomeruli) (7).

EXAMPLE 3

BCG therapy

Evans (Langhurst, UK) or CSL (Parkville, Australia) live freeze dried and attenuated *Mycobacterium bovis* (bacillus Calmette-Gucrin; BCG) vaccine was dissolved in isotomic saline and heat inactivated at 65 C for 45 minutes.

EXAMPLE 4

Mycobacterial subfractions

Mannose-capped lipoarabinomannan (ManLAM) and mycolyl-abarbinogalactan-peptidoglycan complex (MAPG) may be prepared as previously described (8). N-acetylglucosaminyl-N-acetylmuramyl-L-alanyl-D-isoglutamine (GMDP) was purchased from a commercial source (GERBU Biotechnik, Gaibery, Germany). The Applicant acknowledges with appreciation receiving samples of ManLAM and MAPG from the Tuberculosis Repository by Drs P. J. Brennan and

J. T. Belisle through NIAD13 November, 1996, NIH [Contract No. NO1-AU1-25147].

EXAMPLE 5

Random blood glucose estimations

Each mouse was bled by retro-orbital venepuncture of 100-150 μl and the serum glucose concentration measured by the glucose oxidase technique on a Glucostix reagent strip (Ames, Basingstoke, UK). A mouse was considered to be diabetic if it was found to have a random blood glucose level > 11.1mMol/l.

EXAMPLE 6

Haematocrit measurement

Seventy-five microlitres of blood were drawn up into a heparinized capillary tube (Becton Dickinson, Franklin Lakes, NJ, USA) and centrifuged at 1000g for 15 minutes. The height of the column of packed red cells was divided by the total height of the column of blood and expressed as a percentage.

EXAMPLE 7

Direct Coombs test

Mice were bled and the plasma removed. Ten microlitres of packed cells were resuspended in 5ml PBS with 0.3% w/v bovine serum albumin, washed and resuspended in 1ml of the same solution. Triplicates of 100 μl aliquots were placed in 96 well round bottom plates (Nunc, Denmark) with 3 serial ½ dilutions. The plates were vortexed and incubated at 37 C for 1hr. Wells were then assessed for false positive results. Ten microlitres of 10 μg/ml polyclonal goat anti-mouse IgG (Sigma Chemical Company, MO, USA) added. Plates were then vortexed gently and incubated at 37C for a further two hours. Wells in which the cells collected in a button were recorded as negative, while those in which the cells remained spread of the surface over the surface of the well were recorded as positive.

EXAMPLE 8

Assessment of antinuclear antibodies

Sera were assessed for binding to HEp-2 slides (Quantafluor, Chaska, MN, USA). Slides were incubated in phosphate buffered saline (PBS) for 10 minutes. Sera diluted in PBS (starting concentration 1:100) were incubated on the slides at room temperature (RT) for 30 minutes in a moist chamber. Slides were then washed 3 times for 5 minutes in PBS and incubated for 30 minutes at RT with 1:50 FITC conjugated rat anti-mouse Ig (Serotec, Oxford, UK). Slides were again washed 3 times for 5 minutes with PBS, mounted and examined on an Axiophot fluorescence microscope (Zeiss, DDR). Sera from MRL/*lpr-lpr* and BALB/c mice were used

as controls.

EXAMPLE 9

Detection of glomerular immune complex deposits

Kidneys were embedded in Tissue-Tek OCT Compound (Miles, Elkhart, IN) and frozen for histological analysis. Sections of 6 μm were cut on a Microm cryostat (Waldorf, Germany) and mounted on microscope slides, air dried, acetone fixed for 10 minutes and stored at -80C in an air-tight bag containing silica desiccant. When slides were stained, they were thawed to room temperature, fixed in acetone for a further five minutes and blocked with 4% v/v foetal calf serum (FCS; CSL, Melbourne, VIC, Australia). Sections were stained with Goat anti-mouse C3c polyclonal IgG (Nordic Immunological Laboratories, Tilburg, Holland) at a 1:10 dilution in PBS for 45 minutes. Slides were then washed three times in PBS, and coverslipped with polyvinyl alcohol mounting media (Sanofi Diagnostics Pasteur Inc., Chaska, MN) and examined on a Leica fluorescence microscope (Leica Mikoskopie, Postfach, Germany).

EXAMPLE 10

Effects of heat killed BCG on incidence of diabetes

Varying concentrations of heat killed *Mycobacterium bovis* (BCG) were administered intravenously to NOD mice at 0.1 mg, 0.2 mg, 0.4 mg, 0.8 mg, 1.6 mg, 4 mg relative to a control of phosphate buffered saline (PBS; Figure 2). No diabetes was detected in mice given 0.8, 1.6 or 4 mg of BCG (Figure 3). Doses of 0.1 mg, 0.2 mg and 0.4 mg were not completely effective although they did provide some protection.

EXAMPLE 11

Effects of mycobacterial cell wall components on incidence of diabetes

In order to attempt to separate the activity of BCG which prevented IDDM from that which precipitated lupus, the mycobacterial subfractions GMDP, MAPG and ManLAM were tested for these activities. GMDP (10, 5.0, 2.5 and 1.0 μg), ManLAM (50, 25, 12.5 and 6.25 μg), and

MAPG (1.0, 0.5, 0.25 and 0.125 mg) were each suspended in saline and injected intravenously into eight week old female NOD mice. These doses of MAPG and ManLAM were based on estimates of the equivalent quantities in 1mg of BCG, while the does of GMDP was based on that used for immunoadjuvant activity; 10μg being the maximum dose tolerated by mice. Groups of 5 mice were tested and the incidence of diabetes compared to 17 saline treated and 14 BCG treated control mice. The results are shown in Figures 2 to 7.

While 10/17 (59%) PBS treated mice (Figure 2) , 12/20 (60%) of GMDP treated mice (Figure 4) and 13/18 (72%) of ManLAM treated mice developed diabetes (Figure 5) no mice receiving MAPG did so (Figure 6). Haematocrits and Coombs' tests indicated that no mice receiving MAPG developed haemolytic anaemia whereas 11/14 (79%) of BCG treated (0.8-1.6 mg) control mice had a haematocrit below 46% and 7/14 mice were Coombs' test positive. Similarly there was no increase in the expression of antinuclear antibodies in the MAPG treated mice. The effects of mycolic acids administration on the incidence of diabetes is shown in Figure 7.

EXAMPLE 12

Effects of mycobacterial cell wall components on incidence of diabetes

MAPG was injected intravenously into 8 week old NOD mice in doses ranging from 1 mg to 0.125 mg/mouse. All doses tested prevented IDDM. MAPG treated mice were tested for haematocrit, Coombs' test, antinuclear antibodies (ANA) and C3c complement component deposition in the renal glomeruli. No mice developed haemolytic anaemia as detected by lowered haematocrit and positive Coombs' test. ANA levels were not raised above those detected in PBS treated mice. Mild C3c deposition was found in a minority of mice. It was concluded that MAPG administration prevented IDDM without inducing lupus in NOD mice.

It is significant that while MAPG was still effective at preventing IDDM when used at a dose of 0.125 mg/mouse, BCG was ineffective at this dose. This indicates that it is likely that the effect of the MAPG preparation used in these experiments is due to MAPG itself and not to a contaminant from BCG.

EXAMPLE 13

Purification of components of MAPG

Mycobacterium cell envelopes maybe prepared by the method of Azuma *et al* (8) and the soluble glycolipids removed by repeated centrifugation in the presence of SDS. The insoluble envelope component, the peptidoglycan conjugated with mycolic acids-substituted arabinogalactan, is fractionated into its constituent domains by the following procedure.

1. the mycolic acids linkage is cleaved by saponification, base-catalysed methanolysis or ammonolysis and separated from the insoluble residue.

2. the residue is submitted to Smith degradation (periodate, followed by borohydride reduction and mild acid treatment) to cleave the linkage at the rhamnose residue connecting the galactan to the peptidoglycan backbone.

Due to the nature of the furanosyl linkages in the galactan and arabinan domains, these residues are not affected by this treatment. The soluble arabinogalactan is separated from the peptidoglycan by centrifugation.

EXAMPLE 14

Testing components of MAPG

The previous example shows the effectiveness of MAPG in preventing development of diabetes relative to other components of the mycobacterial cell wall. The individual constituents of MAPG are purified according to Example 13 and tested at varying concentrations in NOD mice. The incidence of diabetes is then determined over time.

Those skilled in the art will appreciate that the invention described herein is susceptible to variations and modifications other than those specifically described. It is to be understood that the invention includes all such variations and modifications. The invention also includes all of the steps, features, compositions and compounds referred to or indicated in this specification,

individually or collectively, and any and all combinations of any two or more of said steps or features.

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CLAIMS:

1. A method of immunomodulatory therapy in a mammal said method comprising administering to said mammal an immunomodulating effective amount of one or more components of the cell wall of *Mycobacterium* or a related organism or analogous components from another biological source or chemical equivalents of said components.

2. A method according to claim 1 wherein the immunomodulatory therapy is for the treatment of an autoimmune disease.

3. A method according to claim 2 wherein the autoimmune disease is one or more of IDDM, thyroiditis, atrophic gastritis (type A), pernicious anaemia, Addison's disease, pemphigus vulgaris, pemphigoid, multiple sclerosis, rheumatoid arthritis, systemic lupus erythematosus, discoid lupus erythematosus, haemolytic anaemia, sympathetic ophthalmia, uveitis, idiopathic thrombocytopenia, idiopathic leucopenia, primary biliary cirrhosis, autoimmune chronic active hepatitis, ulcerative colitis, Sjogren's syndrome, dermatomyositis, scleroderma and mixed connective tissue disease.

4. A method according to claim 3 wherein the autoimmune disease is IDDM.

5. A method according to claim 1 wherein the immunomodulatory therapy is for enhancing an anti-tumour immune response.

6. A method according to claim 5 wherein the immunomodulatory therapy is for enhancing an immune response against melanoma or bladder cancer.

7. A method according to any one of claims 1 to 6 wherein the cell wall component comprises mycolyl-arabinogalactan-peptidoglycan (MAPG) or a component thereof or chemical equivalent thereof with or without other associated cell wall components and submolecular components or their chemical equivalents.

8. A method according to claim 7 wherein MAPG is administered in combination with one or more of mycolic acids, peptidoglycan or arabinogalactan or chemical or functional equivalents thereof.

9. A method according to claim 7 wherein MAPG or its components are derived from *Mycobacterium bovis*.

10. A method according to claim 1 wherein the mammal is a human.

11. A method for preventing, delaying onset of, curing, curing in association with islet and/or pancreas transplant replacement or otherwise ameliorating the effects of IDDM in a mammal said method comprising administering to said mammal an IDDM treating effective amount of one or more components of the cell wall of *Mycobacterium* or related organism or analogous components from another biological source or chemical equivalents of said components.

12. A method according to claim 11 wherein the mammal is a human.

13. A method according to claim 12 wherein the cell wall component is MAPG or a derivative or component thereof or their derivatives or chemical equivalents.

14. A method according to claim 13 wherein the MAPG or its derivatives or components is from *M. bovis*.

15. A composition of matter comprising MAPG or a derivative or a component thereof or its derivative or chemical equivalents thereof.

16. A composition according to claim 15 further comprising one or more pharmaceutically acceptable carriers and/or diluents.

17. A method for isolating components of MAPG for use in a therapeutic composition for preventing, delaying the onset of or otherwise ameliorating the effects of diabetes in a mammal

or for use in immunomodulatory therapy said method comprising preparing cell envelopes from a species of *Mycobacterium* or related organism or other suitable biological source, subjecting said cell envelopes to glycolipid removing means to remove soluble glycolipids, treating the product so obtained to break the mycolic acids linkage and isolating said mycolic acids, treating the remaining complex to cleave linkage at rhamnose residue connecting arabinogalactan to the peptidoglycan backbone and separating and isolating arabinogalactan and peptidoglycan.

18. A method according to claim 17 wherein the glycolipids are removed by repeated centrifugation in the presence of sodium dodecyl sulphate (SDS).

19. A method according to claim 17 wherein the mycolic acids linkage is cleaved by saponification, base-catalysed methano lysis or ammonolysis.

20. Use of a cell wall component of *Mycobacterium* in immunomodulatory therapy.

21. Use according to claim 20 wherein the immunomodulatory therapy is for the treatment of diabetes.

22. Use according to claim 20 wherein the immunomodulatory therapy is for the treatment of carcinoma.

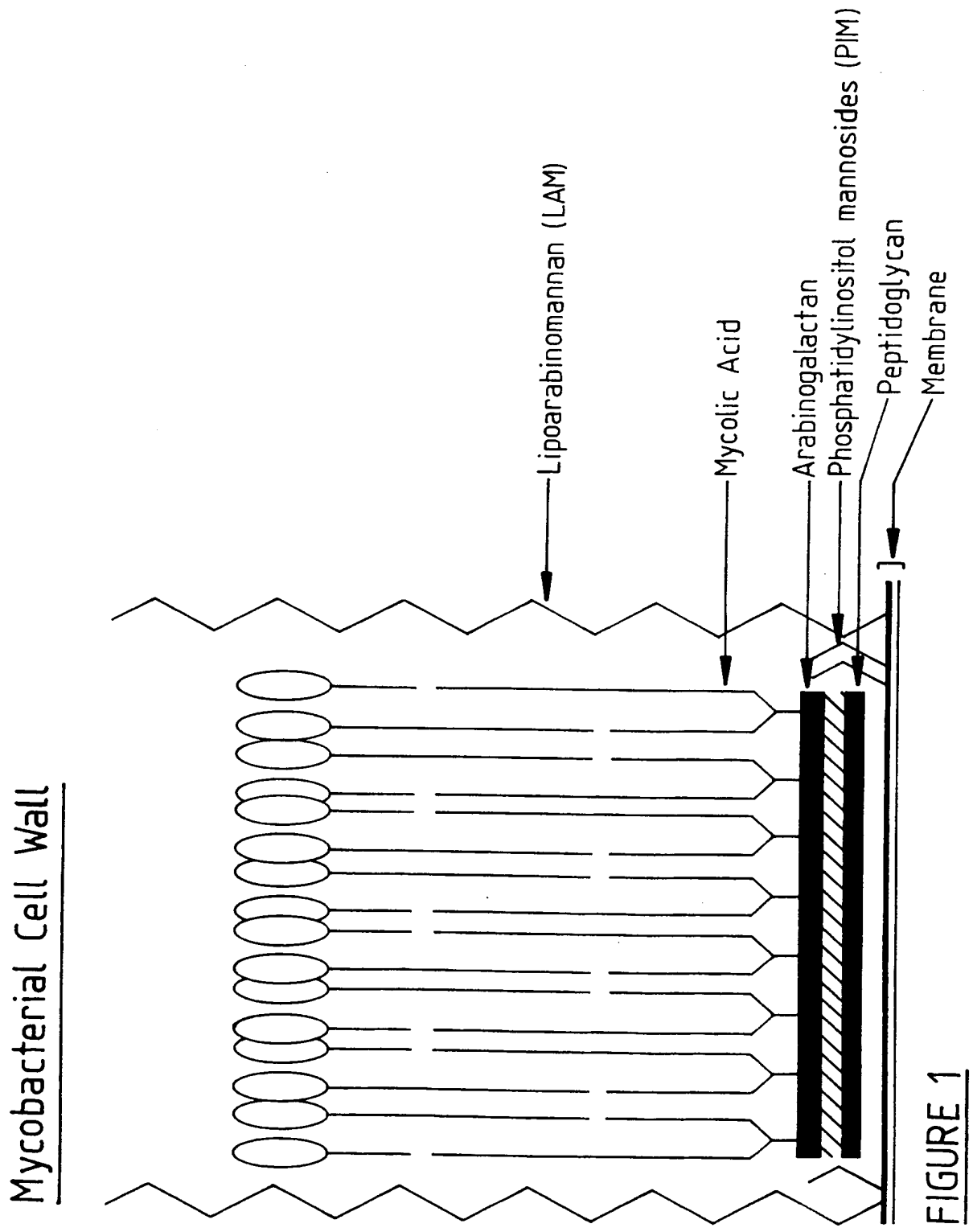

Mycobacterial Cell Wall
Lipoarabinomannan (LAM)
Mycolic Acid
Arabinogalactan
Phosphatidylinositol mannosides (PIM)
Peptidoglycan
Membrane

FIGURE 1

FIGURE 2

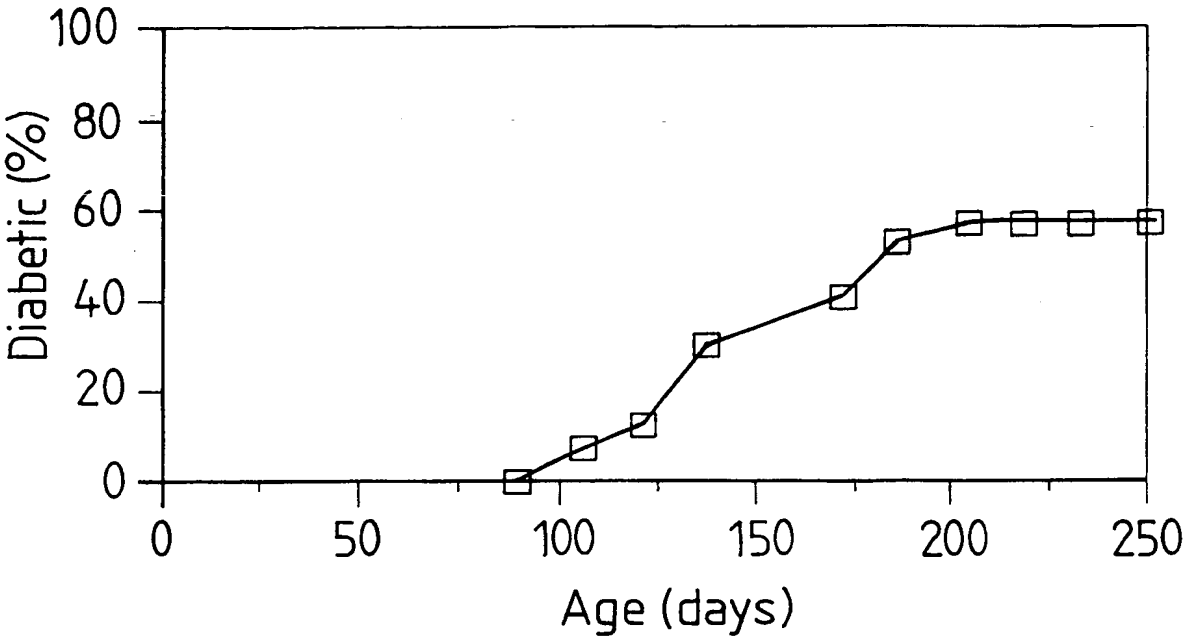


FIGURE 3

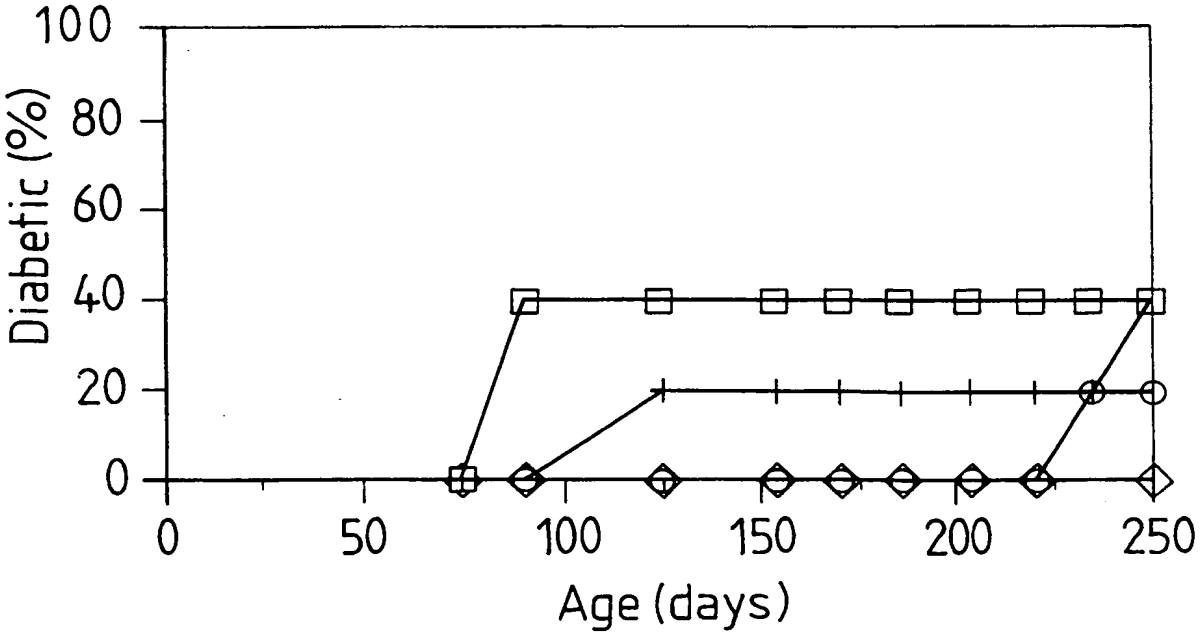


FIGURE 4

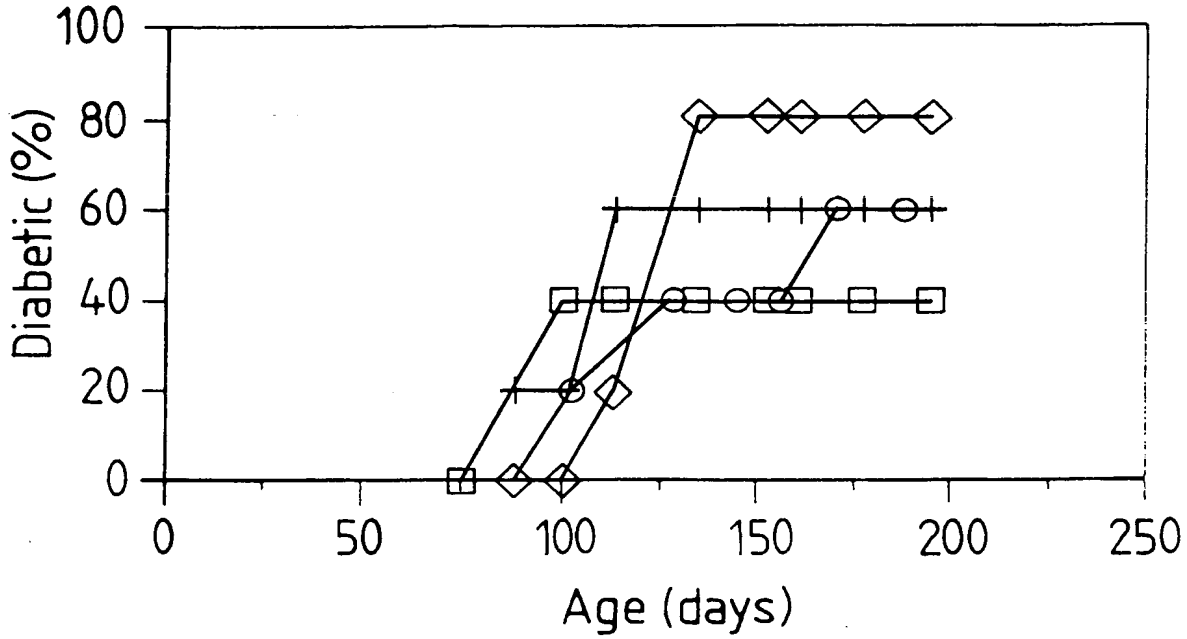


FIGURE 5

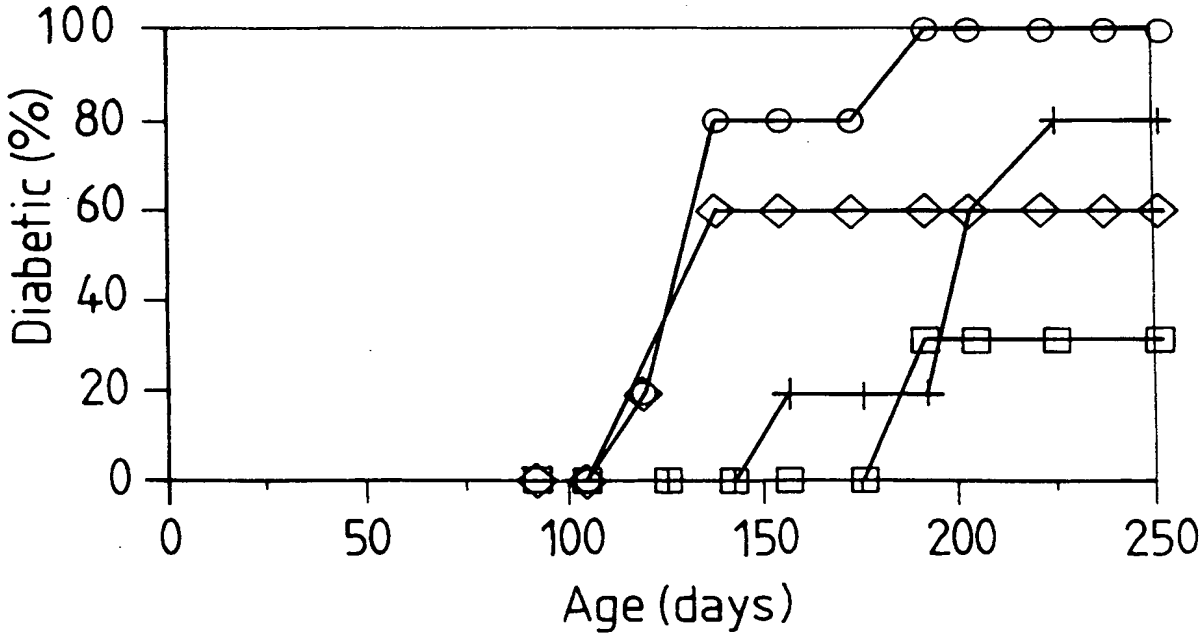


FIGURE 6

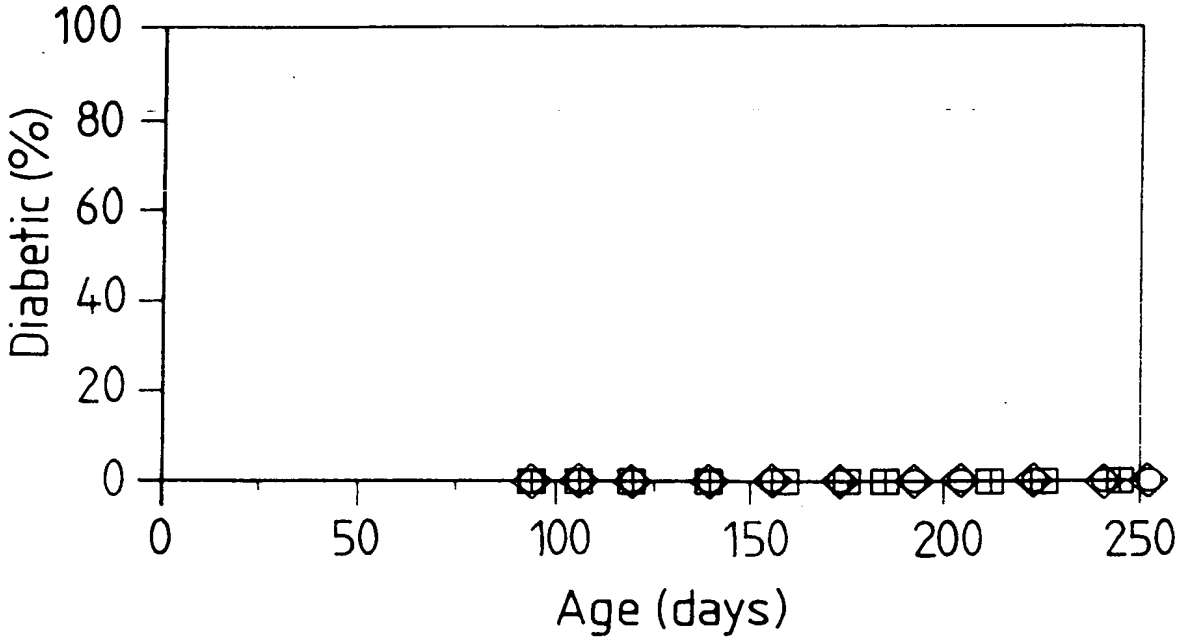
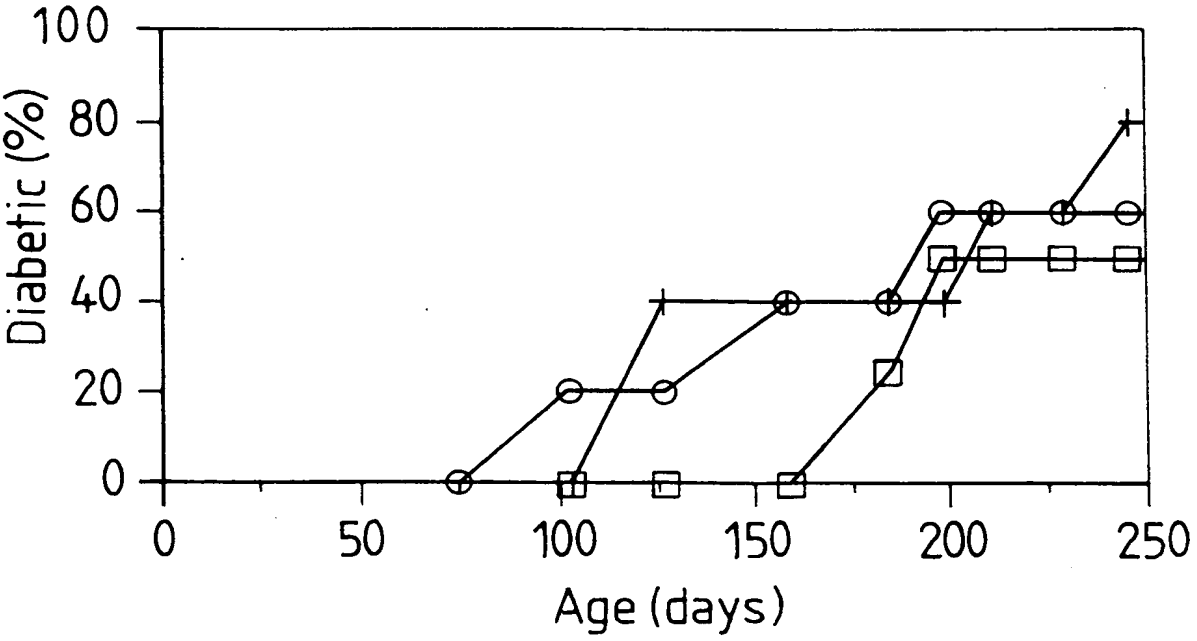


FIGURE 7



INTERNATIONAL SEARCH REPORT

International Application No.
PCT/AU 97/00770

A. CLASSIFICATION OF SUBJECT MATTER

Int Cl[6]: A61K 39/04, 38/02, C07K 14/35

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC: A61K C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
AU; IPC as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
DERWENT: (MYCOBACTER: or BCG) AND (CELL()WALL or IDDM or DIABET: or AUTOIMMUNE or AUTO()IMMUNE or CANCER); MAPG
CHEMICAL ABSTRACTS: ?PEPTIDOGLYCAN? AND (MYCOLYL or MYCOLYLARABINO); MAPG

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 96/00579 A (LAVESARZNIMITTEL GMBH) 11 January 1996 entire document	1-6, 10-12, 20-22
X	WO 96/26288 A (ADCOCK INGRAM LIMITED) 29 August 1996 entire document	1-17, 20-22
X	WO 94/16727 A (VETREPHARM, INC.) 4 August 1994 entire document	1-6, 10-12, 20-22

[X] Further documents are listed in the continuation of Box C

[X] See patent family annex

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search
18 December 1997

Date of mailing of the international search report
13 JAN 1998

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INTERNATIONAL SEARCH REPORT

International Application No.
PCT/AU 97/00770

C (Continuation) **DOCUMENTS CONSIDERED TO BE RELEVANT**

Category*	Citation of document, with indication, where appropriate, of the relevant passages *(Remove spaces when completed if the page is too long)*	Relevant to claim No.
X	WO 87/02249 A (RAGLAND William L) 23 April 1987 entire document	1-6, 10-12, 20-22
X	GB 2120548 A (RIBI IMMUNOCHEM RESEARCH INC.) 7 December 1983 entire document	1-17, 20-22
X	US 4152423 A (ADAM et al.) 1 May 1979 entire document	1-6, 10-12, 20-22
X	Hirschfield GR *et al.*, "Peptidoglycan - Associated Polypeptides of *Mycobacterium tuberculosis*" *J. Bacteriol.* (Feb 1990) 172(2),1005-1013 abstract	1-22
X	Azuma I *et al.*, "Fractionation of Mycobacterial Cell Wall" *J. Bacteriol.* (Nov 1968) 96(5), 1885-1887 entire document	15-19
X	Baxter A.G. *et al.*, "Mycobacteria precipitate an SLE-like syndrome in diabetes-prone NOD mice", *Immunology* (1994) 83, 227-231 abstract and discussion	1-16, 20-22
X	Sadelain M.W.J. *et al.*, "Prevention of Type I diabetes in NOD mice by adjuvant immunotherapy", *Diabetes* (May 1990), 39(5), 583-587 abstract	1-16, 20-22
X	Harada M *et al.*, "Prevention of overt diabetes and insolitis in NOD mice by a single BCG vaccination" *Diabetes Res. Clin. Pract.* (1990) 8, 85-89 abstract	1-16, 20-22
X	Mediline Abstract UI: 93171635, Qin MY *et al.*, "Complete Freund's adjuvant-induced Tcells prevent the developmentand adaptive transfer of diabetes in nonbese diabetic mice", *J. Immunol* (1993), 150(5), 2072-2080 abstract	1-16, 20-22
X	Mediline Abstract UI: 93209598, Chugh IB *et al.*, "Protective efficacy of different cell-wall fractions of Mycobacterium tuberculosis", *Folia. Microbiol (Praha)*, (1992), 37(6), 407-412 abstract	1-16, 20-22
X	Mediline Abstract UI: 83254632, Beaudet R *et al.*, "Stimulation of non-specific anti-tumor resistance in the mouse using cell wall preparations from four BCG substrains",. *Ann. Immunol. (Paris)* (1983), 134C(2), 215-226 Abstract	1-16, 20-22
X	Dockrell HM *et al.*, "Inductiion of Th1 Cytokine Responses by Mycobacterial Antigens in Leprosy", *Infect. Immun..*, (Oct 1996), 64(10), 4385-4389 Abstract	1-16, 20-22
X	McNeil M *et al.*, "Evidence for the Nature of the Link between the Arabinogalactan and Peptidoglycan of Mycobacterial Cell Walls" *J. Biiol. Chem.* (25 Oct 1990) 265(30), 18200-18206	15-19
X	Barnes PF *et al.* "Tumour Necrosis Factor Production in Patients with Leprosy" *Infect. Immun.* (Apr 1992), 60(4), 1441-1446 abstract	1-16, 20-22

INTERNATIONAL SEARCH REPORT
Information on patent family members

International Application No.
PCT/ AU 97/00770

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report		Patent Family Member					
WO	9600579	AU	28884/95	CA	2194138	CZ	9603753
		DE	4422859	EP	804211	NO	965593
		PL	317892				
WO	9626288	AU	47287/96	CA	2187779	EP	811075
		EP	755517	PL	316792	WO	9528642
WO	9416727	AU	59680/94	BR	9406496	CA	2154689
		CN	1118574	EP	681479		
WO	8702249	AU	65258/86	CA	1293190	EP	238657
		US	4744984				
GB	2120548	AU	14015/83	CA	1202903	NZ	204020
		US	4520019				
US	4152423	AR	192504	AU	48697/72	BE	791472
		CA	1013691	CH	564085	DE	2256838
		ES	408766	FR	2160326	JP	48061619
		LU	66491	NL	7215627	US	4036953
		AU	57069/73	CA	1009972	CH	587346
		FR	2189019	GB	1440842	JP	49066817
		ZA	7304076				

END OF ANNEX

INTERNATIONAL SEARCH REPORT

International Application No.
PCT/AU 97/00770

C (Continuation) **DOCUMENTS CONSIDERED TO BE RELEVANT**

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X	Baxter A.G. *et al.*, "Mycobacteria precipitate an SLE-like syndrome in diabetes-prone NOD mice", *Immunology* (1994) 83, 227-231 abstract and discussion	1-16, 20-22
X	Sadelain M.W.J. *et al.*, "Prevention of Type I diabetes in NOD mice by adjuvant immunotherapy", *Diabetes* (May 1990), 39(5), 583-587 abstract	1-16, 20-22
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X	Mediline Abstract UI: 83254632, Beaudet R *et al.*, "Stimulation of non-specific anti-tumor resistance in the mouse using cell wall preparations from four BCG substrains",. *Ann. Immunol. (Paris)* (1983), 134C(2), 215-226 Abstract	1-16, 20-22
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INTERNATIONAL SEARCH REPORT

International Application No.
PCT/AU 97/00770

A. CLASSIFICATION OF SUBJECT MATTER

Int Cl[6]: A61K 39/04, 38/02, C07K 14/35

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC: A61K C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
AU; IPC as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
DERWENT: (MYCOBACTER: or BCG) AND (CELL()WALL or IDDM or DIABET: or AUTOIMMUNE or AUTO()IMMUNE or CANCER); MAPO
CHEMICAL ABSTRACTS: ?PEPTIDOGLYCAN? AND (MYCOLYL or MYCOLYLARABINO); MAPO

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 96/00579 A (LAVESARZNIMITTEL GMBH) 11 January 1996 entire document	1-6, 10-12, 20-22
X	WO 96/26288 A (ADCOCK INGRAM LIMITED) 29 August 1996 entire document	1-17, 20-22
X	WO 94/16727 A (VETREPHARM, INC.) 4 August 1994 entire document	1-6, 10-12, 20-22

[X] Further documents are listed in the continuation of Box C

[X] See patent family annex

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

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